THE BASAL METABOLIC RATE OF THE AMERICAN NEGRO, WITH PARTICULAR REFERENCE TO THE EFFECT OF MENSTRUATION ON THE FEMALE

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THREE FIGURES

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A review of the literature on the determination of the effect of the menstrual cycle on basal metabolism of women yields conflicting evidence. The most general agreement seems to be on the existence of the premenstrual rise with a lowering during the actual menstruation and in the immediately postmenstrual period. This has been emphasized by Snell, Ford and Rowntree ('20), Rowe and Eakin ('21), and Wakeham ('23). Similar relationships have been reported by Kunde ('23), Collett and Liljestrand ('24), Hafkesbring and Collett ('24), Benedict and Finn ('28), Gustafson and Benedict ('28), Rogers and Flemming ('28), Hitchcock and Wardwell ('29), Udaondo ('32), Conklin and McClendon ('30), Wible ('31), and Arvay and Meyer ('32).

Other investigators have concluded that there is no demonstrable effect. Chief among these may be listed Salomon ('05), Zuntz ('06), Gephart and DuBois ('16), Wiltshire ('21), Blunt and Dye ('21), Lantz ('25), and Sandiford, Wheeler and Boothby ('31).

The work of Zuntz was done on two subjects only, while Gephart and DuBois reported three tests on a single subject. The validity of these results may be questioned from the paucity of data submitted. Wiltshire reported conflicting results on five subjects. The low values in metabolic rate during menstruation reported by Blunt and Dye were considered within the range of variation that may occur at any time. Lantz also reported negative results from a study of a healthy woman, while Sandiford, Wheeler and Boothby found no change in heat production, which can be considered indicative of a fundamental change in the intensity of the oxidation processes.

This study aimed at determining the periodic variation (if any) in the basal metabolic rate of Negro women during the menstrual cycle and at ascertaining whether their values differ enough from the standard value for white North Americans to suggest a racial factor in basal metabolism.

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EXPERIMENTAL PROCEDURE

One hundred and eighty-two basal determinations were made on twenty-seven healthy women between the ages of 17 and 35 years, inclusive. All tests were carried out on subjects in the post-absorptive state, after a night's rest in the metabolism laboratory. A Benedict-Roth-Collins metaboler with a kymograph attached was used in all cases. All calculations were made from the graphic record of the respiration of the subjects. In the computations, the DuBois height-weight formula was used for surface area determinations. For normal standards the Boothby and Sandiford modified form of the DuBois normal standards was used.

Tests were made before, during and after menstruation with an average interval of 7 days, except for two subjects for whom daily variations are reported. Thirty basal metabolism determinations were also made on fourteen Negro men between the ages of 18 to 24 years, inclusive.

RESULTS

Data for all subjects with respect to the average basal metabolic rate expressed as percentage above or below normal and the number of tests yielding this average are given in table 1.

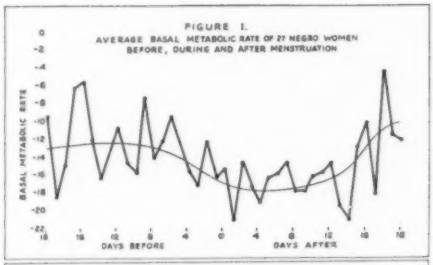
TABLE 1

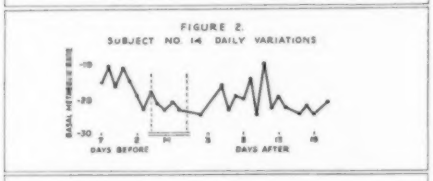
Basal metabolism of Negro women and men compared with the DuBois standards as modified by Boothby and Sandiford.

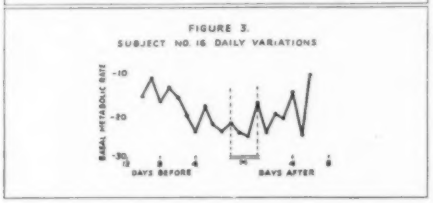
WOMEN						MEN		
Subject	No. of tests	Comparative average basal metabolic rate	Subject	No. of tests	Comparative average basal metabolic rate	Subject	No. of tests	Comparative average basal metabolic rate
1	6	— 11.9	16	23	— 13.34	1	2	— 9.2
2	5	— 16.64	17	6	— 7.2	2	3	— 16.3
3	9	— 14.73	18	9	— 14.72	3	2	— 3.2
4	5	— 13.96	19	7	— 15.0	4	2	— 9.7
5	5	— 11.88	20	4	— 13.32	5	2	— 11.8
6	5	— 19.56	21	2	— 7.66	6	2	— 3.6
7	3	— 8.43	22	3	— 4.31	7	2	— 8.7
8	5	— 4.65	23	3	— 17.13	8	2	— 15.3
9	5	— 13.32	24	5	— 22.06	9	2	— 11.8
10	8	— 18.02	25	4	— 9.96	10	2	— 11.8
11	3	— 20.1	26	6	— 6.56	11	2	— 16.0
12	5	— 15.48	27	4	— 14.43	12	2	— 20.8
13	8	— 19.65				13	2	— 11.2
14	26	— 18.62				14	2	— 22.3
15	8	— 21.6						

Figure 1 represents a composite smoothed frequency polyogon for all female subjects in which the average daily values were plotted against the basal metabolic rate before and after menstruation. Individual daily variations on two subjects are given in figures 2 and 3.

Pulse, temperature and blood pressure are not recorded here, as they were within normal limits.







DISCUSSION OF RESULTS

The results herewith presented indicate that Negro women give a lower basal metabolic rate than the value reported for white women of North America. The average deviation from the DuBois standards as modified by Boothby and Sandiford was — 14.6%.

Dr. Alberta Iliff, writing on behalf of Dr. Robert C. Lewis and his associates, of the University of Colorado School of Medicine, states (unpublished communication):

"We have made determinations of basal metabolism on men and women between the ages of 15 and 40 years. Although the number of tests is too few to make a statistical analysis, the mean values were definitely lower than the standards of Boothby, Berkson and Dunn

(Am. J. Physiol., vol. 116, p. 468, 1936). Consequently, the Department of Biochemistry has recently adopted standards of reference which are the Boothby, Berkson and Dunn standards less 10%. These revised standards agree well with the results which we have found."

If these observations should prove to be generally valid, it would indicate that the basal metabolic rates of Negroes which we have determined are not significantly lower than those of white subjects.

In the selection of the male subjects, care was used in order to ensure a varied group, such as athletes in training, manual workers on the school farm, and students not engaged in any extra-curricular activity.

Considering the type of subjects, the co-operative spirit, and the number of tests made on each subject, it is felt that the data represent the true metabolic level as nearly as could be desired. The reported effects of the menstrual cycle on basal metabolism were confirmed. These consist essentially of a pre-menstrual rise in basal metabolism with a lowering during actual menstruation and in the immediately post-menstrual period. This is shown in the composite graph (fig. 1) for all subjects in which the average daily values were plotted against the basal metabolic rate before and after menstruation.

SUMMARY

1. The basal metabolic rates of twenty-seven women and fourteen men were determined by the Benedict-Roth-Collins metabolism apparatus and found to be 14.8 and 12.44%, respectively, below the Boothby and Sandiford modified form of the DuBois standards.

2. The reported effects of the menstrual cycle on basal metabolism (a pre-menstrual rise with a lowering during the actual menstruation and in the immediately post-menstrual period) were confirmed.

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM IN THE CHICK

III. SOME TIME RELATIONSHIPS IN THE ACTION OF VITAMIN D [1]

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The mode of action of a number of the vitamins is now comparatively well understood. Nevertheless, the mode of action of vitamin D remains obscure. The literature on this subject has been reviewed by Reed, Struck, and Steck ('39) with these conclusions: (1) The primary function of vitamin D is to render calcium and phosphorus available to the tissues, (2) it also makes the tissues more receptive to these ions, and (3) the manner in which this is accomplished remains to be elucidated.

More recently Nicolaysen and Jansen ('39) demonstrated that there was no difference in the density of calcification in albino rats whether they received vitamin D or not, provided that adequate amounts of calcium and phosphorus were actually brought to the blood. There was, however, some histological difference in the structure of the bones of the animals which received vitamin D. Cohn and Greenberg ('39), using P^{32} as a trace element, concluded that vitamin D acts by aiding the conversion of organic to inorganic phosphorus. Morgareidge and Manly ('39), also using P^{32} as a trace element, concluded that the action of vitamin D is not limited to the control of the intestinal absorption of calcium and phosphorus.

Since the manner in which vitamin D acts still remains understood only in a very general way, it was felt by the authors that it might be worthwhile to make studies of mineral retentions following single oral dosages of vitamins D_2 and D_3 to the chick, with particular reference to these considerations: (1) How soon after the vitamin dosages an improvement in mineral balances becomes evident, (2) how long the favorable effect of the dosages persists, and (3) whether there is any quantitative relationship between the level of mineral retention and the amount of vitamin D available to the chicks at different times. It was

[1] Presented before the Division of Biological Chemistry of the American Chemical Society at Cleveland, April 4, 1944.

also hoped that the information so obtained might reveal significant differences in the action of vitamins D_2 and D_3 which would aid in explaining the fact that the former is only about 2 to 3% as effective as the latter in the chick. Previous papers of this series (McChesney, '43 b, '43 c) have shown that this phenomenon may be accounted for in part by the better absorption of vitamin D_3, but even after allowance for this is made, the vitamin D_3 remains about twenty times as effective as D_2.

Studies of the type outlined above have not been possible thus far because of the lack of definite information about (and methods for the study of) the metabolism of vitamin D in various species. Knudsen, Remp, and Barlow ('41) studied the metabolism of several forms of vitamin D in the albino rat and found that after 10 consecutive days of oral dosage, only a fractional part of 1 day's dosage could be recovered from the carcasses on the eleventh day, indicating very rapid excretion and/or inactivation. McChesney [2] has studied the metabolism of massive (hypercalcemic) doses of vitamins D_2 and D_3 in the chick. The former was found, under certain conditions, to disappear from the organism at a rate considerably exceeding that of the latter.

OUTLINE OF EXPERIMENTAL PROCEDURES

Male white Leghorn chicks were received in the laboratory on the second day of life. They were immediately offered water and a vitamin D-free chick ration, which is slightly modified from that of Hart, Kline, and Keenan ('31); i.e., 2 parts of corn oil are substituted for 2 parts of yellow corn. On the sixth day of life the chicks were divided into three groups of twenty, and one group of forty. The purpose was to use the first three groups for mineral balance studies, and the last for studies of vitamin D_2 metabolism. Accordingly, for the first three groups, the food consumption was now determined, the excreta were collected, and distilled water was substituted for tap water. At first the intervals studied were 3 or 4 days in duration, but later in the experiment, when it was desirable to study short-time variations, periods as short as 1 day were used. The chicks were weighed at the beginning and end of each period for which the mineral balances were to be determined. Food and excreta were analyzed for calcium and phosphorus by methods which have been outlined previously (McChesney, '43 b); the diet was found to contain 0.86% Ca, 0.68% P. From the data so obtained the calcium and phosphorus balances were calculated in terms of milligrams

[2] To appear in Proc. Soc. Exp. Biol. and Med.

retained per 100-gm. chick per day. The weight factor was introduced in order to eliminate the effect of growth as the experiment proceeded.

On the twentieth and thirty-fourth days of life the chicks were given oral supplements (by stomach tube) as follows: Group A, propylene glycol, 0.2 ml.; group B, vitamin D_3, 60 I.U. in 0.2 ml. propylene glycol; group C, vitamin D_2, 2400 I.U. in 0.2 ml. propylene glycol; group D (40 chicks) same as group C.

Metabolism of vitamin D_2. At selected intervals after the vitamin supplementations (table 1) groups of four chicks were taken from group D for vitamin D assay. Since these chicks were on the same regimen as group C, it was considered that their average body content of vitamin D would be the same as that existing in group C at the same time. The four chicks were at once digested in boiling alcoholic KOH, and extracts

TABLE 1

Average body weights and mineral retentions of chicks receiving oral supplements of vitamin D at selected intervals.

OBSERVATION PERIOD, DAYS OF LIFE	GROUP A			GROUP B			GROUP C			BODY CONTENT OF VITAMIN D_2 [3, 4]
		Retention [2] of			Retention of			Retention of		
	Av. wt.[1]	Ca	P	Av. wt.	Ca	P	Av. wt.	Ca	P	
7–10	71	68.6	46.3	71	90.0	51.4	71	81.5	46.2	
11–14	90	37.2	28.2	89	46.4	36.6	90	56.4	39.8	
15–17	102	26.7	20.0	103	24.0	22.0	106	30.6	28.3	
18–20	110	24.6	18.9	115	29.6	29.0	118	22.3	22.3	
21 [5]	117	29.0	17.1	122	39.5	28.0	126	35.4	24.5	625
22	119	30.3	17.8	126	73.4	45.6	131	63.3	41.2	787
23	119	23.6	16.0	131	55.8	32.8	136	52.8	31.8	...
24–25	123	15.2	15.2	138	54.5	39.6	144	46.1	35.1	460(25)
26–27	130	20.8	18.6	150	47.1	35.3	158	34.3	25.9	365(27)
28–29	135	19.2	16.0	159	34.7	30.8	170	26.3	23.8	...
30–31	138	17.5	14.1	166	25.0	18.8	180	24.9	20.6	262(30)
32–34	145	23.8	19.4	179	19.4	18.1	192	21.8	18.3	...
35 [5]	151	25.0	21.4	188	37.9	27.2	201	44.4	34.1	1050
36	153	21.0	17.4	191	44.5	32.1	208	36.4	24.2	...
37	156	15.6	14.4	197	52.4	35.2	212	34.8	24.5	567
38–39	160	12.0	10.3	212	43.4	31.6	221	31.1	23.3	435(39)
40–41	164	12.4	11.8	224	24.5	20.5	235	27.6	23.8	470(41)
42–43	168	8.6	7.3	231	19.3	15.7	241	23.1	16.7	...
44–45	169	7.6	6.5	246	21.2	17.5	254	18.3	14.7	325(45)

[1] Grams.

[2] Calculated as milligrams of the element retained per 100-gm. chick per day.

[3] Of group D, which received the same treatment as group C.

[4] The figures in parentheses indicate the day of life on which the observations were made where such would otherwise be ambiguous.

[5] Vitamin supplements given at the beginning of this 24-hour period as follows: Group A, none; group B, 60 I.U. of vitamin D_3; group C, 2400 I.U. of vitamin D_2.

were prepared according to methods already described (McChesney, '43 a). The ether extract so obtained was evaporated and taken up in a volume of oil such that the concentration of vitamin D was estimated to be 16.7 I.U. per milliliter. (These volumes varied from 120 ml. 24 hours after supplementation down to 15 ml. on the tenth day after supplementation.) Then an equal volume of an oily solution of vitamin D_2 known to contain 16.7 I.U. per milliliter was added. This was necessary in order to guarantee sufficient material to complete the bioassays in case the first test fell outside the readable range. This procedure, however, has the effect of doubling the experimental error when the final estimate of the vitamin content of the chicks is made.

In the case of the 7- and 10-day interval extracts, it was impossible to reduce the ether solutions to the desired volumes by evaporation, and the preparations so obtained were not homogeneous. The volume of known vitamin D solution added was the same as originally planned, but the final volume of the mixture instead of being 30 ml., for example, was as much as 95 ml. Thus, in the bioassay it was necessary to administer as much as 0.3 ml. per rat per day (by stomach tube) instead of the usual 0.1 ml. The material appeared to be somewhat irritant, and the animals did not eat well. This resulted in some "starvation cures", and the results for the 7- and 10-day intervals after supplementation may be somewhat high.

Unfortunately the prophylactic dose of vitamin D_3 is entirely too small to permit a parallel study of its metabolism. Even 24 hours after administration sufficient material could not be extracted to permit a bioassay.

RESULTS AND DISCUSSION

The numerical data are given in table 1.

Even before any vitamin supplements were given, some variability in the mineral balances of the different groups was evident. However, the observations for days 15 through 20 were about as uniform as could be expected, and the trend indicated that a "resistance level" had been reached. This is confirmed by the fact that group A, which received no vitamin D at any time, maintained essentially this level of mineral retention through the thirty-sixth day of life. The most reasonable interpretation of the behavior of the birds through the preliminary control period (days 6–20) is that they inherited a considerable supply of vitamin D, and that this permitted them to maintain satisfactory mineral balances through the first 14 days of life. By this time they had apparently exhausted all but a small part of the inherited D and were

unable to maintain mineral balances above the resistance level. Only after a long time (group A, days 37–45) was the resistance level permanently broken; the birds then went into terminal rickets and deaths began to occur. A reasonable interpretation of this would be that during the 14- to 36-day interval they still had available a very small amount of vitamin D which they conserved tenaciously, but which was finally exhausted.

The first supplementation with vitamin D_2 or D_3 produced only a slight improvement in mineral balances within the first 24 hours. However, this cannot be interpreted to mean that there is a latent period before vitamin D becomes effective since part of the excreta collected for this period represents food ingested during the preceding 24 hours; furthermore, the second vitamin supplementation did produce a more evident immediate improvement. The positive effect from the first supplementation lasted 8 days, when the resistance level had again been reached. The duration of the positive effect from the second supplementation was barely 7 days, provided the same resistance level is taken as a reference point rather than the value observed in the control chicks at that particular time.

The effect of the second supplementation was quantitatively inferior to the first in terms of mineral balances. This seems logical in view of the fact that the supplements were the same in both cases, but in the meantime the chicks had increased in weight by about 80%.

In view of the previously mentioned differences in rates of inactivation of vitamins D_2 and D_3 in the chick, it was thought that some characteristic difference in the mineral retention curves for the two products might be found. (For example, following administration of vitamin D_2 the same total retention might be accomplished in a shorter period of time.) However, there is nothing in the character of the data to indicate that vitamin D_2 and D_3 affect mineral retention differently either qualitatively or quantitatively, barring the fact that forty times as much of the former is required to produce the same quantitative effect. With both preparations positive retentions of about the same magnitude were observed for the same time interval after supplementation. The results, therefore, offer no clue as to the cause of the remarkable difference in activities of the two preparations in the chick.

From the amounts of vitamin D_2 found in the chicks 24 hours after the administration of 2400 I.U., the amount which was actually absorbed may be calculated roughly. The figure for day 21 is clearly too low, as a value of around 900 I.U. would fit in better with the rest of the data. Extrapolating back to day 20, it would appear that a value of about

1000 I.U. should have been found immediately following absorption. Of this amount about 250–300 I.U. may be assumed to have been already available to the chicks since they were then at what has been termed the "resistance level" of mineral retention (as they were on day 30 when the body content of vitamin D was 262 I.U.). Therefore, roughly 700–750 I.U. or 30% of the dose administered was absorbed. The body content of 1050 I.U. on day 35 indicates the same general conclusion, with allowance for a carry-over of about 250 I.U. from the first supplementation. However, it must be kept in mind that this seemingly large amount of 250 I.U. represents only 5 I.U. in terms of natural vitamin D, or less than 2 days' requirement for a chick.

The vitamin D was inactivated at a rate of 10 to 15% per day during the time that its metabolism was studied. This rate of inactivation is materially less than has previously been found for massive doses of the vitamin.[3]

The mineral balances, taking the expected daily variations into account, are in general directly related to the amount of vitamin D available at the time. The most interesting phenomenon revealed by the work is the minimum level of calcium and phosphorus retention (about 20 mg. per 100-gm. chick per day) which the birds were able to maintain for a considerable period of time without receiving any vitamin D. This could, of course, be due to an extremely small amount of vitamin D in the diet, an amount which finally became completely inadequate when the birds became quite large.

SUMMARY

The baby chicks used in this study had a supply of vitamin D adequate for about 14 days of life. Their calcium and phosphorus retentions then reached a level of about 20 mg. per 100-gm. chick per day, a level which they were able to maintain for 3 weeks. After this there was a decline to very low levels as the birds went into a state of terminal rickets. However, chicks which received oral supplements of vitamin D_2 or D_3 showed an immediate increase in calcium and phosphorus retentions. The improvement was evident for 7 or 8 days, after which the chicks returned to the same low level of retention. A second vitamin supplementation of the same magnitude produced a positive, but quantitatively inferior, effect, probably due to the intervening growth of the birds. Following supplementation, the increased level of mineral retention was directly related to the amount of vitamin D available. The body content of vitamin D_2 following an oral dose of 2400 I.U. increased to about

[3] See footnote 2, p. 230.

1000 I.U., and, in the ensuing 10 days, fell to about 250 I.U. This residue, equivalent to about 6 I.U. of vitamin D_3, appeared to be rather tenaciously conserved.

ACKNOWLEDGMENT

The technical assistance of Donald Seppelin and Mrs. Charles Miller is hereby gratefully acknowledged.

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STUDIES ON THE COMPARATIVE NUTRITIVE VALUE OF FATS

V. THE GROWTH RATE AND EFFICIENCY OF CONVERSION OF VARIOUS DIETS TO TISSUE IN RATS WEANED AT 14 DAYS[1]

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TWO FIGURES

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There have been several reports in the literature from the Wisconsin group that butterfat possesses certain saturated long-chain fatty acids, not present in vegetable oils, which accentuate the growth of young rats during the first several weeks following weaning although the variation in growth response may disappear after 6 weeks (Schantz, Elvehjem and Hart, '40; Schantz, Boutwell, Elvehjem and Hart, '40). In a later study (Boutwell et al., '43a), it was reported that this particular superiority in growth-promoting property of butterfat over corn oil is only to be noted in those diets where the sole carbohydrate is lactose. This superiority of butter disappeared when lactose was replaced by glucose, sucrose, dextrin or starch, and actually slightly greater gains (though not statistically significant ones) were noted under these circumstances during the 6-week interval following weaning. In an extension of this work (Boutwell et al., '43b) with synthetic diets where the lactose or other carbohydrates made up 48% of the diet instead of 32% used in the earlier tests, no differences were noted between butter or lard and a series of vegetable fats when a mixture of carbohydrates was employed; however, in most cases, poorer growth was obtained when lactose alone was used. With seven types of oleomargarines of animal and vegetable origin, the growth during a 6-week period averaged 6.4% lower than butter when the high lactose was employed and was identical with it when the mixed carbohydrate diet was fed.

[1] This work was carried out under a research grant from The Best Foods, Inc. The authors wish to acknowledge the helpful advice of Prof. Anton J. Carlson of the University of Chicago, of Prof. Arthur W. Thomas of Columbia University, and of Dr. H. W. Vahlteich of The Best Foods, Inc., during the course of the experiments.

In a previous report from this laboratory (Deuel et al., '44), no differences were noted in the growth of 21-day weanling rats fed a diet where lactose was the sole carbohydrate at the level found in milk, irrespective of whether the fat added to the mineralized skimmed milk powder was a butter, a margarine, or corn, cottonseed, olive, peanut or soybean oil, all of which were fortified with vitamins A, D and E. The ratios of food consumed to increase in body weight were similar when the different diets were employed, which would indicate a similar efficiency in utilization. It was also found (Deuel and Movitt, '44) that rats prefer a butter diet or one flavored with diacetyl or commercial butter flavor to one where this flavor is not present. It was suggested that superior growth response of animals fed on a butter diet over those on other fats which are unflavored in experiments where ad libitum feeding is employed may well be ascribed to a greater food consumption.

Another condition where the superiority of butterfat over the vegetable oils has been reported to be enhanced, is in rats prematurely weaned (Boutwell et al., '43a). The Wisconsin investigators found that the variations in growth response had practically disappeared in rats which were not started on the diet until they were 30 days of age; on the other hand, they were accentuated in rats weaned at 14 days over those weaned at the usual 21-day period. These results are interpreted as indicating that the rat gradually stores the growth-promoting compounds (saturated long-chain fatty acids) during nursing as well as when fed the stock ration so that an adequate reserve is available when these substances later are withheld from the diet. It is suggested that it is difficult to deplete rats of this factor when they have had access to the stock ration for 10 days. Zialcita and Mitchell ('44), however, have failed to confirm the superiority of butterfat over corn oil on the subsequent growth of rats weaned as early as 7 days and fed in both cases until 21 days of age, an artificial liquid milk diet containing butterfat or corn oil followed by a synthetic solid milk diet with these fats thereafter. The present tests also are designed to give further information on the relation of age to nutritive value of the fat with rats weaned at 14 days of age.

EXPERIMENTAL

The tests were carried out essentially the same as in the earlier studies (Deuel, Movitt, Hallman and Mattson, '44), using the same strain of rats. In order that the rats be sufficiently developed to make possible a survival after weaning at 14 days, the growth was stimulated

by reducing the litters to two rats at 3 days of age. When the litters were 7 days old, the mothers which had previously received our stock diet were placed on the butter, margarine, corn oil, cottonseed oil, peanut oil or soybean oil diet described below. When the young were weaned, one was placed on one of the six diets listed above and the litter mate was assigned to the butter diet as a control. In the case of the group where the mothers had been placed on the butter diet, the second rat in the litter was placed on the cottonseed oil diet. During the first week cotton batting was placed in their cages. The temperature of the room was maintained throughout at about 72°C. Small Petri dishes were employed for feeding during the first week. Because of the spilling of the food during the first week, it was not possible to obtain accurate values of the amount eaten until the second week. Practically all of the rats survived and gained on an average of 5 to 8 gm., which is probably less than they would have had they remained with their mothers.

Experiments were completed on 120 male rats and 112 female rats from our stock colony. In any tests where diarrhea developed, both litter mates were discarded. The diet differed from that used for series II but was identical with that used for series III (diet 53 previously listed as diet II) in which whole butter or whole margarine was used instead of the separated fat, and additional water was added to the oil diets. The following proportions of mineralized skimmed milk powder and vitamin-fortified fats were used: Diet 54, skimmed milk powder 64.5 parts, oil or fat 26.9 parts, water 8.6 parts (caloric value 4817 Cal. per kilo.); diet 53, skimmed milk powder 70.6 parts, whole butter or whole margarine 29.4 parts (caloric value 4690 Cal. per kilo.). Care was taken to use only fresh fats. Diets were prepared weekly, and stored in a refrigerator; they were fed ad libitum. The diets were flavored with commercial butter flavor.[2] The present tests are designated as series IV.

RESULTS

The averaged weights of the rats receiving the different diets are illustrated in figure 1 for the males and in figure 2 for the females. The values are shown for the start (14 days) for the 1st, 3rd, 6th and 12th week thereafter. The weights of the rats (listed as series III) which received similar diets but which were weaned at 21 days (Deuel, Movitt, Hallman and Mattson, '44) are included for comparison at corresponding ages.

[2] B. F. A. supplied by Verlay Products Corporation, 1621 Carroll Ave., Chicago, Ill., was used in a ratio of 4 parts per 1,000,000 parts of fat.

TABLE 1

The average weight increases, food consumption, caloric intake and efficiencies in male and female rats weaned at 14 days.

DIET	AVERAGE OVER 2ND TO 12TH WEEK INCLUSIVE						AVERAGE EFFICIENCY ((GM. GAIN/CAL. CONSUMED) × 100)) OVER FOLLOWING WEEKS INCLUSIVE							
	Weight increase [1]		Total food		Total calories		Male rats				Female rats			
	M	F	M	F	M	F	2–3	4–6	7–12	2–12	2–3	4–6	7–12	2–12
	gm.	gm.	gm.	gm.										
Butter	235.9 ± 14.9	145.6 ± 3.8	934	721	4385	3380	9.8	5.3	4.48	5.38	10.8	5.6	2.35	4.32
Cottonseed oil	244.1 ± 5.1	143.3 ± 3.8	818	690	3940	3325	10.5	7.5	4.80	6.19	9.5	5.1	2.74	4.32
Corn oil	234.4 ± 5.1	143.0 ± 3.3	808	665	3890	3205	9.8	7.8	4.44	6.03	10.4	5.8	2.43	4.45
Butter	226.9 ± 8.8	143.2 ± 4.0	879	703	4120	3300	9.3	6.9	4.15	5.52	9.9	5.5	2.28	4.34
Cottonseed oil	247.5 ± 10.1	141.0 ± 5.3	838	694	4030	3340	10.0	8.1	4.55	6.14	9.0	5.1	2.74	4.22
Butter	256.3 ± 10.0	148.0 ± 4.7	922	733	4320	3300	10.0	7.2	4.43	5.93	9.6	5.5	2.35	4.48
Margarine	230.8 ± 4.4	137.2 ± 5.1	884	708	4150	3320	9.2	7.2	4.22	5.57	10.0	5.0	2.28	4.13
Butter	234.6 ± 5.8	137.6 ± 5.3	885	735	4155	3445	9.7	6.6	4.57	5.64	9.2	5.2	2.18	3.99
Peanut oil	234.7 ± 10.6	138.1 ± 2.8 [2]	831	704 [2]	4000	3390 [2]	10.5	6.8	4.48	5.87	8.9	4.7	2.61 [2]	4.08 [2]
Butter	237.6 ± 6.8	157.9 ± 7.0 [2]	889	747 [2]	4170	3505 [2]	9.7	6.3	4.48	5.69	9.8	5.6	2.61 [2]	4.51 [2]
Soybean oil	244.8 ± 5.4	156.6 ± 6.3 [3]	812	678	3910	3265	11.0	8.0	4.40	6.23	9.5	6.2	2.92	4.79
Butter	233.7 ± 6.8	145.4 ± 5.8 [3]	854	688	4010	3220	9.8	6.3	4.36	5.83	10.5	5.4	2.62	4.52
Average butter tests	237.5	145.8			4190	3360	9.7	6.5	4.42	5.67	10.0	5.5	2.38	4.35
Average non-butter tests	239.4	143.0			3990	3310	10.2	7.6	4.72	6.50	9.6	5.3	2.50	4.33

The first diet listed in each group of two was the original diet of the mother before the rats were weaned.

[1] Including the standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of experiments.

[2] Eight experiments only.

[3] Eight experiments only with females throughout.

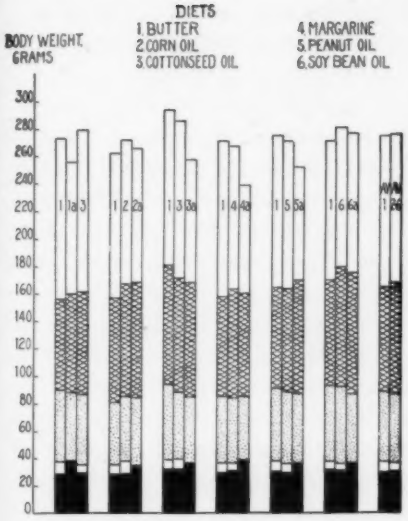

Figure 1

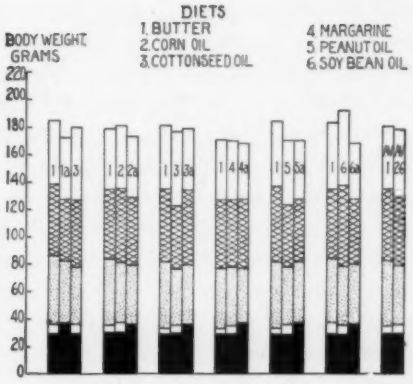

Figure 2

Fig. 1 The average body weight of male rats at weaning (14 days of age for series IV, 21 days of age for series III, designated by letter "a") in solid block, at 21 days (series IV only) to top of lower blank space, at 35 days (stippled), at 56 days (cross-hatched) and at 98 days (to top of upper blank space). Averages are for ten rats in all cases with series IV and for twelve to fourteen rats in various groups in series III. The average butter and average non-butter series (at extreme right) are for sixty rats each.

Fig. 2 The average body weight of female rats at weaning (14 days of age for series IV, 21 days of age for series III, designated by letter "a") in solid block, at 21 days (series IV only) to top of lower blank space, at 35 days (stippled), at 56 days (cross-hatched) and at 98 days (to top of upper blank space). Averages are for ten rats in all cases with series IV except experiments on peanut and soybean oils (eight rats each), and thirteen to sixteen rats in various groups in series III. The average butter and average non-butter series (at extreme right) are for fifty-six rats each.

The total gain in weight, food consumption and the caloric equivalent of the food are summarized for the 11-week period from 21 to 98 days of age. Because of the unavoidable spilling of food during the first week, the values for this period are not included. The efficiency of conversion of ingested food to body tissue is compared on the basis of the gain in weight per calories consumed. These data are summarized in table 1.

DISCUSSION

No differences were noted over a 12-week period in the growth rate of rats weaned at 14 days of age when placed on a diet containing mineralized skimmed milk powder and fat, irrespective of whether the latter

was a butter, a margarine or such vegetable oils as corn, cottonseed, peanut or soybean, all of which had been fortified with vitamins A, D and E. Thus, the average total increase in weight of the male rats receiving the various diets was 244.7 gm. for the butter controls (sixty animals), and 245.5 gm. for the same number of litter mates which received one of the five other fats in place of butter. The corresponding increments in growth of the female rats were 151.7 and 149.0 gm., respectively, for fifty-six rats receiving the butter diet and the same number which ate the other fat diets. Although there are some variations in the average total gain in weight of the animals receiving the various fats and their butter diet controls, the differences are in no case significant statistically. In some cases the differences favor the vegetable oil group, while in some instances the opposite is true. As shown in figures 1 and 2, no appreciable variations were observed in growth rate between the groups receiving the various fats and butter at 1, 3 or 6 weeks. According to the results of Schantz, Elvehjem and Hart ('40), it is at these earlier periods that such differences should be noted.

The efficiency with which the food is utilized also shows no clear-cut differences between the butter diets and those containing the other fats. The over-all average for the males covering 11 weeks is 0.0567 and 0.0650 gm. gain per Calorie of butter diet and non-butter diet, respectively, that was consumed; for the females the corresponding values are 0.0435 and 0.0433 gm., respectively. The ratios between increase in body weight and caloric intake show approximately a similar relationship for the 2-3, 4-6 and 7-12 week periods.

As we have noted earlier (Deuel, Movitt, Hallman and Mattson, '44), the efficiency of conversion of ingested food to body tissue is less in females than in males and in both sexes becomes lower as the animals become larger. This is, of course, explained by the fact that the efficiencies are calculated on the gross energy consumption rather than on the net value after subtraction of that used for basal metabolism, specific dynamic action, etc. As the proportion of the total used for growth becomes smaller, the efficiency based on the gross intake will also become smaller. Furthermore, in the later periods the rate of growth becomes less, and the efficiency approaches a value of zero when growth ceases. This disproportion between caloric needs for basal requirements and growth accounts for the lower efficiency in the females where the growth rate is lower than the males in the later periods.

Although the reason for the difference in efficiency in our tests and those of Boutwell et al. ('43b) is not entirely clear, it may possibly be

because of the lower proportion of lactose in our diet. Whereas these investigators report that the efficiency of corn oil was 31% less than butter during the first 3 weeks, in our tests it averaged 105% of the efficiency of the butter controls.

A lower absorption with a decreased efficiency would result if a diarrhea is present. In the few cases where diarrhea occurred in our tests, the experiments on both litter mates have been excluded from consideration, as they were in all probability from the results of the Wisconsin investigators. When the lactose content of the diet was increased to as high as 73%, we have found not only that a diarrhea may occur but also in many instances it may terminate fatally (Ershoff and Deuel, '44). Another possible explanation may be a strain variability in the animals used. Whereas weanling rats of the Long-Evans strain died on an average of 4.9 and 11.5 days after receiving diets containing 73.2% of beta-lactose or lactose (Ershoff and Deuel, '44), the average survival time for the University of Southern California strain was 22 days for beta-lactose and 26 days for the lactose group. Also, a similar variation in the period of incidence of a characteristic alopecia was noted between these two strains. In view of this one might also expect a variation with the Sprague-Dawley rats, the strain used by the Wisconsin investigators.

No marked variations in the average body weights are to be noted between the prematurely weaned rats and those of series III which were reported earlier. This would indicate that no harmful effects resulted from the shortening of the lactation period.

According to these results, aside from differences in vitamin content, corn, cottonseed, peanut and soybean oils and a margarine and a butter have essentially equal growth-promoting values when fed with lactose as the exclusive carbohydrate in the proportion found in milk. In confirmation of the work of Zialcita and Mitchell ('44) such a relationship also occurs in prematurely weaned rats.

SUMMARY

In experiments on 120 male and 112 female rats, the rate of growth of animals weaned at 14 days was found to be identical over a period of 12 weeks on diets of mineralized skimmed milk powder to which was added vitamin-fortified corn, cottonseed, peanut or soybean oil or a margarine as on similar diets containing butter as the fat. The efficiency of transformation of these diets to body tissue was also similar within experimental error.

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STUDIES ON CAROTENOID METABOLISM

V. THE EFFECT OF A HIGH VITAMIN A INTAKE ON THE COMPOSITION OF HUMAN MILK [1]

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The amount of vitamin A present in human milk is regulated by the diet in much the same way as in the case of cow's milk. Thus, on a vitamin A-low diet the milk may contain an insufficient amount of vitamin A to prevent avitaminosis. In fact, Thatcher and Sure ('32) have reported the death of an infant, who had been fed exclusively on breast milk from a malnourished mother, and in whom frank symptoms of vitamin A deficiency were apparent before death as well as at autopsy. On the other hand, milk from women on an average American dietary has been shown by Macy et al. ('27) to contain amounts of vitamin A sufficient to permit normal growth and reproduction in rats on an A-free diet supplemented with 2.5 or 3.0 ml. of pooled human milk daily. Dann ('36) found that although the vitamin A content was of the same order of magnitude in human and cow's colostrum, the vitamin A in human milk far exceeded that in cow's milk, the average vitamin A activity in 104 samples calculated for both carotene and vitamin A being 346 I.U. per 100 ml.

Although it is evident that the presence of adequate amounts of vitamin A in the diet will cause the transfer of vitamin A to the milk, there is a wide range of intake over which no parallel increase in vitamin A excretion is to be noted in the human as well as the cow. For example, McCosh, Macy, Hunscher, Erickson and Donelson ('34) found no rise in the vitamin A in the milk of three women who took 15 gm. of cod-liver oil daily in addition to an abundant and well-chosen diet. Moreover, there was no evidence of a deleterious effect on the fat content of milk as had previously been found to be the case for cows (Goldring et al.,

[1] This investigation was made possible through grants from the Nutrition Foundation, Inc. We also wish to thank the Vitamin Oil Producers Institute for furnishing the vitamin A capsules.

'26). Dann ('36) also found no increase in the vitamin A content of colostrum or of mature milk in women whose diets were supplemented with cod-liver oil starting during pregnancy and continuing during the lactation period, but the dosages of cod-liver oil are not indicated. The failure to obtain an appreciable augmentation in the vitamin A in milk under such dietary regimes has been interpreted to mean that there is some factor which limits the amount of vitamin A which may pass into milk (Dann, '32).

However, the administration of what may be considered massive doses of vitamin A in the form of shark-liver oil to Guernsey and Holstein cows already receiving an excellent diet has been shown to result in a marked increase in the vitamin A content of milk (Deuel et al., '41, '42). In some cases the vitamin A of the butter fat was found to exceed 300 I.U. per gram in contrast to control values of about 40 I.U. per gram. Moreover, no decrease either in milk production or in the fat content of the milk was observed under these circumstances. In later work from this laboratory, similar effects were found on the vitamin A content of the hen's egg as a result of the administration of large amounts of this vitamin to hens (Deuel et al., '43). In both species of animals the quantity of vitamin A so transferred was roughly proportional to the dosage of vitamin A after the threshold had been exceeded; it was not until vitamin A in excess of 500,000 I.U. daily had been administered to cows and in excess of 3300 I.U. per day was fed to chickens that any increased vitamin A was to be found in the milk or eggs.

The present studies were designed to determine whether the human subject would react in a similar manner to dosages of vitamin A which are considerably higher than those usually regarded as adequate. By analogy from the cow experiments it was believed that an intake of 100,000 I.U. daily would be the level which should cause an increase in excretion of vitamin A in the milk of lactating women. Although there are some instances where toxic effects have been reported in animals when large dosages of vitamin A have been given over long periods, the results of Straumfjord ('42) where daily dosages of 100,000 I.U. and higher were given to patients in some cases continually over a period of 4 years without deleterious effects, would indicate that these levels could be used safely in women. Straumfjord ('40) also has given similar doses during 6 months or more of pregnancy where a beneficial effect on vernix caseosa was noted. These clinical reports as well as the earlier experiments on cows and chickens where considerably higher levels of vitamin A were administered over a number of months were taken as evidence that the dosages employed here were in the therapeutic range.

EXPERIMENTAL

The experiments were carried out on forty-two women who received vitamin A in amounts of 50,000, 100,000 or 200,000 I.U. each.[2] In addition, experiments were made on nine women who received vitamin A in a dosage of 200,000 I.U. daily for only 7 to 10 days prior to obtaining the sample. A total of 127 samples were obtained from these subjects. In most cases the vitamin A supplementation was started at 6 months of pregnancy and continued until lactation was voluntarily terminated.[3] The patients selected were Caucasian from the middle or upper economic bracket who had the desire to nurse their offspring and who had adequate breast tissue to make this possible. All of these patients were directly under the care of one of us (B.J.H.) during the time that this work was being carried on.

In addition, eighty-five samples of milk were obtained from normal patients in various stages of lactation who were not receiving any vitamin A other than that obtained in an adequate diet. These are listed as control experiments. All samples were obtained by breast pump. The samples were immediately brought to the laboratory, refrigerated and the vitamin A determination made on the following day. Vitamin A and carotene were in most cases determined in duplicate on the non-saponifiable residue. This was carried out as follows: after mixing 10 ml. of milk, 10 ml. of 95% ethanol and 2 ml. of 40% KOH, the mixture was saponified by refluxing for 30 minutes. It was then transferred to a continuous all-glass extraction apparatus with sufficient 50% alcohol to make a total volume of 80 ml. This was extracted with low-boiling Skelly Solve for 3 hours. All operations were carried out in a dark room.[4] After washing the petroleum ether three times with a total of 50 ml. of 1% HCl, it was dried overnight with anhydrous Na_2SO_4, filtered, evaporated to small volume on a water bath and the last traces of petroleum ether removed in the vacuum oven.[5] The residue was dissolved in 3 ml. of dried redistilled $CHCl_3$ and vitamin A determined by the Carr-Price method on a Klett-Summerson photoelectric colorimeter using a special 600-mμ. filter. Readings were made 1 minute after mix-

[2] Prepared by Gelatin Products, Inc., Detroit, for the Vitamin Oil Producers Institute.

[3] The subjects were instructed to take the capsules daily but we cannot be certain that in all cases they were conscientious in taking their supplements regularly. Deviations in this may account for some of the variations obtained.

[4] The light bulbs were covered with a special lacquer which prevented any destruction of vitamin A. It was kindly furnished us by Mr. A. Cherkin of Don Baxter and Co., Glendale, Calif.

[5] The presence of traces of alkali in the non-saponifiable residue presumably causes clouding of the $SbCl_3$ reagent. Also, it was found that the presence of a trace of petroleum ether would also prevent the satisfactory application of the Carr-Price method. Our procedures satisfactorily cleared up these difficulties.

ing with the $SbCl_3$ reagent, and vitamin A calculated from a standard curve which had been made using similar procedures on standard reference cod-liver oil. Correction was made for the effect of carotene. Carotene was determined on the combined $CHCl_3$ extracts in the same colorimeter using the blue filter (420 mμ.).

In order to determine whether the administration of the supplementary vitamin A was accompanied by changes in other constituents of the milk, estimations were also made on protein, fat, ash and total solids. Protein was determined by the usual macro-Kjeldahl procedure, fat by the Babcock method and total solids and ash by the usual methods.

RESULTS

The results on vitamin A and carotene are summarized in table 1.

The vitamin A content in the non-supplemented group averaged 331 I.U. as preformed vitamin A per 100 ml. or 424 I.U. when the activity of the carotene is included during the period 2 to 10 days following parturition but not including samples of colostrum. In the later periods the total vitamin A averaged about 270 I.U. per 100 ml. of milk. The averages of Dann ('32) made at a number of periods during lactation are intermediate between these although this investigator reports an average value of total vitamin A of 632 I.U. per 100 ml. for human colostrum (111 samples).

In general, progressively higher values were obtained with the increasing doses of vitamin A, the values for the 2- to 10-day period being 599, 869, and 1047 I.U. for the 50,000, 100,000, and 200,000 I.U. supplement groups, respectively. In all cases these values are statistically higher than that for the control. In general, there is a decrease in vitamin A parallel to that in the control group in the later periods with all levels of vitamin A intake. In most of the cases in groups 3 and 4, the differences are marked and are statistically significant where there were enough observations for such statistical treatment. However, in group 2 the values although considerably higher than the controls are statistically significant only in the first period.

The maximum value for vitamin A of 2160 I.U. per 100 ml. was found in milk obtained during the 2- to 10-day period from a patient in group 4. Values almost as high (1992 and 1746) were obtained in the milk of patients in group 3.

Even though in all groups the quantities of vitamin A in the milk tend to fall in the 11- to 30- and 31- to 60-day periods as compared with that found in the 2- to 10-day period, they are somewhat higher in the last period. There is no evidence, therefore, that the continued adminis-

TABLE 1

The vitamin A and carotene in human milk of subjects who had received supplementary vitamin A from the sixth month of pregnancy and of controls who had received no supplementary vitamin A.

PERIOD POST-PARTUM	VITAMIN A IN I.U.[1] IN GROUP				CAROTENE IN MICROGRAMS [1] IN GROUP				TOTAL VITAMIN A IN I.U. IN GROUP			
	1: Control	2: 50,000 I.U.	3: 100,000 I.U.	4: 200,000 I.U.	1	2	3	4	1	2	3	4
days												
2–10	331 ± 21	599 ± 87	869 ± 121	1047 ± 181	56 ± 5	89 ± 12	101 ± 21	78 ± 15	424	747	1037	1177
		3.01	**3.90**	**3.93**								
	(40)	(16)	(15)	(11)								
11–30	232 ± 25	471 ± 114	498 ± 96	871 ± 123	43 ± 3	34 ±5	38 ± 6	32 ± 6	304	528	561	928
		2.05	**2.67**	**4.22**								
	(8)	(8)	(8)	(8)								
31–60	171 ± 21	325 ± 117	459 ± 74	501 [2]	38 ± 7	24 ± 6	40 ± 6	30 [2]	234	365	526	551
		1.29	**3.75**									
	(13)	(6)	(8)	(5)								
61 +	216 ± 30	289 ± 14	795 ± 121	660 [2]	39 ± 6	33 ± 4	54 ± 9	35 [2]	281	344	885	718
		2.15	**4.66**									
	(24)	(16)	(15)	(2)								

The figures in parentheses are the number of observations in the average. The numbers in average are similar in corresponding groups in carotene and total vitamin A columns.

The bold face figures are the ratio of mean difference to standard error of mean difference when compared with control group. When this exceeds 3.0, the results are considered significant.

[1] Including the probable error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

[2] Too few observations for a statistical evaluation.

tration of the large doses of vitamin A results in its greater destruction with a lowered amount being secreted in the milk. For example, the vitamin A excretion remained high in subject AF over a period of 6 months as shown by the following analyses for vitamin A in I.U. per 100 ml. of milk: 1452 on the 6th day, 840 on the 42nd, 1296 on the 73rd, 1392 on the 90th, 1746 on the 135th and 1032 on the 180th day.

TABLE 2

The average total solids, protein, fat and ash in the milk of women receiving no supplementary vitamin A (group 1), 50,000 I.U. (group 2), 100,000 I.U. (group 3) or 200,000 I.U. (group 4) daily at various intervals during lactation.

GROUP AND SAMPLE	SAMPLE NO.	NO. OF SAMPLES [1]	TOTAL SOLIDS	PROTEIN	FAT	ASH
			%	%	%	%
Group 1	1	40	12.15 ± 0.13(a)	1.81 ± 0.11(c)	2.7 ± 0.4	0.304 ± 0.026(a)
Control	2	8	12.58 ± 0.40(a)	1.50 ± 0.13(a)	3.5 ± 0.6	0.233 ± 0.036(a)
	3	13	11.44 ± 0.50	1.30 ± 0.15	2.5 ± 0.5	0.175 ± 0.063
	4	24	12.27 ± 0.45(a)	1.27 ± 0.04(a)	3.8 ± 0.4	0.209 ± 0.050(a)
Group 2	1	15	11.59 ± 0.21	2.10 ± 0.22	2.4 ± 0.3	0.330 ± 0.082
50,000 I.U.	2	8	11.92 ± 0.46(a)	1.47 ± 0.15	3.3 ± 0.4	0.252 ± 0.023(a)
	3	5	12.20 ± 0.64	1.40 ± 0.01	3.1 ± 0.5	0.254 ± 0.072
	4	16	11.50 ± 0.29(c)	1.10 ± 0.09(c)	3.0 ± 0.4	0.211 ± 0.011(c)
Group 3	1	14	11.32 ± 0.58(a)	2.05 ± 0.14	3.1 ± 0.3	0.313 ± 0.015(a)
100,000 I.U.	2	8	12.69 ± 0.39(a)	1.54 ± 0.56	3.3 ± 0.3	0.265 ± 0.019(a)
	3	8	12.40 ± 0.45	1.39 ± 0.04	4.3 ± 0.7	0.224 ± 0.009
	4	15	14.36 ± 0.82(a)	1.19 ± 0.12	5.5 ± 0.7	0.198 ± 0.050(a)
Group 4	1	11	12.13 ± 0.32(a)	2.17 ± 0.19(a)	2.9 ± 0.4	0.325 ± 0.019(a)
200,000 I.U.	2	8	12.72 ± 0.57	1.59 ± 0.13	3.9 ± 0.5	0.238 ± 0.045
	3 [2]	5	12.57	1.58	3.9	0.288
	4 [2]	2	12.48	1.40	4.4	0.205

Sample 1, 2–10 days after parturition: sample 2, 11–30 days; sample 3, 31–60 days; sample 4, 61 days plus.

[1] Number of fat samples. When the number of determinations considered in calculating the average was 1 less, they are marked with "a"; 2 less "b"; 3 less "c"; 5 less "e".

[2] Too small number for statistical evaluation.

There is also a marked tendency for milk carotene to reach considerably lower levels in the periods following the one immediately after parturition. There are no consistent differences between the various groups with respect to milk carotene.

Table 2 gives a summary of the total solids, protein, fat and ash content of the various samples.

No differences were observed in the protein or ash contents obtained at comparable periods of lactation irrespective of whether the subjects were receiving vitamin A or not. There is a progressive drop in protein as lactation progresses as was noted by Holt, Courtney and Fales ('15) as well as by Bell ('28); a similar decrease in ash content was found as reported by the former investigators. On the other hand, there would appear to be a slightly higher fat content in groups 3 and 4 during the last two periods when compared with the control group. In general the fat content steadily increases as lactation progresses, as proved by Bell ('28).

DISCUSSION

The administration of vitamin A in large doses effectively increases the vitamin A content in the milk of the lactating human subject in the same way as in the cow. Although a statistically significant rise was observed in the early milk after the administration of 50,000 I.U. of vitamin A daily, the increase was not maintained in the later samples. On the other hand, the administration of 100,000 I.U. of this vitamin daily more than doubled the vitamin A content of the milk and this augmentation was maintained for at least 6 months which was the maximum length of time that the studies were continued. The failure of previous workers (McCosh, Macy, Hunscher, Erickson and Donelson, '34; Dann, '36) to demonstrate this rise in vitamin A content of milk after ingestion of moderate amounts of vitamin A is readily explainable because the level of the dose administered was too low. As we have shown earlier with the cow (Deuel et al., '41), there is a considerable range over which no increase in vitamin A occurs. Thus, no increase was noted until 700,000 I.U. per day were fed; at levels of 235,000 and 470,000 I.U. daily the results were completely negative. In the human the threshold would appear to be in the neighborhood of 50,000 I.U. daily. As the quantity administered is increased above this amount, the quantity excreted is also gradually increased.

In order to test how quickly the women would respond to vitamin A therapy, a supplement of 200,000 I.U. was given daily to nine women who were 3–7 months post-partum and who had previously received no supplementary vitamin A. The average value of vitamin A in the milk 7 to 10 days after the vitamin A was started was found to be 452 I.U. per 100 ml. which is almost double the control value of 216 I.U. In one case (L.), the rise was from 27 to 774 I.U.

There is no clear-cut demonstration of a decrease in content of milk carotene associated with the increased vitamin A ingestion similar to the effect previously found in the cow (Deuel et al., '41; '42) and in the

eggs of chickens (Deuel et al., '43). This may be an example of a species difference which is quite widespread in the metabolism of the carotenoids (Zechmeister, '37). On the other hand, it was impossible to control the diets in the case of the experiments on human subjects, and it it is conceivable that any depressing effect on carotene excretion which otherwise might have been observed may have been obscured and counteracted by the variations in the quantity of carotene ingested.

The administration of the large doses of vitamin A was not attended by any marked alterations in other components of the milk. Thus, the deleterious effects exhibited by a decreased fat content of the milk noted by Goldring et al. ('26) and others in the cow after the administration of large amounts of cod-liver oil were absent here, as we have noted earlier when shark-liver oil and vitamin A concentrates were given to cows. In the present tests, there is not only no evidence of a decreased content of fat, but there is an increase in most instances where the vitamin A is administered although this was not found to be a statistically significant rise. Also, there is apparently no alteration in the proportion of other components in the milk. There is a progressive lowering in protein and ash and a rise in fat content as the lactation cycle proceeds in the confirmation of earlier published results of other workers.

There is no evidence of any deleterious effects of the vitamin A on the mothers or offspring. The babies without exception grew well. Although in most cases, lactation was voluntarily terminated in several months, in one case it was continued satisfactorily for 6 months and the milk production was not diminished over what would be expected in women receiving no supplementary vitamin A. We have, however, failed to confirm the report of Straumfjord ('40) on the effect in decreasing vernix caseosa, although this may be explained by the fact that the period of vitamin A supplementation was limited only to the last trimester rather than the last two trimesters of pregnancy.

SUMMARY

1. The average amount of vitamin A in the milk of women receiving no supplementary vitamin A was 331 I.U. per 100 ml. for the 2- to 10-day period, 232 I.U. for the samples collected between the eleventh and thirtieth days, 171 I.U. for the 31- to 60-day samples and 216 I.U. for the samples collected after 2 months.

2. The administration of vitamin A in amounts of 50,000 I.U. daily starting with the sixth month of pregnancy caused a statistically sig-

nificant increase in the early milk but the significant difference was not maintained in the later periods; after 100,000 I.U. the vitamin A was doubled and the increase was obtained throughout. After the ingestion of 200,000 I.U. per day the values were over three times the level in the unsupplemented group.

3. The maximum level obtained was 2160 I.U. per 100 ml. during the 2- to 10-day period following parturition in the group receiving 200,000 I.U. daily.

4. No changes were noted in protein, fat or ash content or total solids by supplementation with vitamin A. Protein and ash decreased progressively and fat increased as the lactation cycle proceeded.

5. No depression in carotene excretion similar to that previously noted in cows and in chickens concomitantly with the administration of large doses of vitamin A was noted in the present tests.

6. There is no evidence of a deleterious effect caused by the continued administration of the large dosages of vitamin A, employed in the present tests, to women during the last trimester of pregnancy and during the lactation period. On the other hand, these data should not be interpreted to mean that the feeding of large doses of vitamin A to pregnant and lactating women is either necessary or desirable.

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THE RELATIONSHIP OF GLYCERIDE STRUCTURE TO FAT DIGESTIBILITY

I. SYNTHETIC GLYCERIDES OF STEARIC AND OLEIC ACIDS [1]

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INTRODUCTION

In recent years considerable research has been directed toward the study of fat digestibility. Most of the work has been focused on the relative coefficients of digestibility of various natural and hydrogenated fats and oils that are used in edible products. The ultimate purpose of such research should be, and in the main has been, a better knowledge of the fundamental principles of fat digestion and metabolism.

There is, among others, one basic question as yet unanswered. What is the single factor, or combination of factors, which limits the digestibility of certain fats? In 1915, Langworthy and Holmes, in a study with human subjects, concluded that "of those fats tested, the fats of low melting point are capable of more complete assimilation than those of high melting point". From later work Lyman ('17) decided that the melting point was not the only, and probably not the chief factor determining the rate of hydrolysis and absorption. Hoagland and Snider ('42) were unable to find a consistent relationship between melting point and digestive coefficient. Nor were they able to correlate digestibility with per cent of saturated acids in the fat or with linoleic acid content. These results have been corroborated by results obtained at the University of Pittsburgh (Longenecker et al., '44).

There have been, however, several instances where stearic acid and its esters have been found to be poorly digested. As early as 1890 Arnschink found tristearin only 9–14% digestible in dogs, with nearly all the undigested stearin being found in the feces as triglyceride. On the other hand, Lyman ('17) found that dogs utilized 95% of the tripalmitin and 82% of the palmitic acid that were present in their diets. Hoagland and Snider ('43) also found tristearin and stearic acid to be

[1] Presented before the Division of Biological Chemistry of the American Chemical Society in New York City, September, 1914.

poorly absorbed, while tripalmitin and palmitic acid were much more digestible.

It would appear, then, that further elucidation of the limiting factors of digestibility of fats would result from additional experiments with synthetic glycerides and mixtures of glycerides. In the present communication digestibility studies have been conducted with diets containing synthetic mono-oleodistearin, mono-stearodiolein, and 2:1 and 1:2 mixtures of triolein and tristearin.

EXPERIMENTAL

Preparation of glycerides

Oleic acid was prepared by fractional crystallization of the methyl esters of red oil,[2] according to the method outlined by Brown ('41), followed by saponification and acidification of the purified methyl oleate. The acid chloride was prepared by the reaction of oleic acid with oxalyl chloride (Wood et al., '44), followed by fractional distillation. Stearoyl chloride was obtained by refluxing stearic acid [3] with thionyl chloride, and distillation of the resulting product. Both acid chlorides were water white.

The simple triglycerides were synthesized by direct esterification of the fat acids with equivalent amounts of dry glycerol, with about 1% of p-toluene sulfonic acid present as catalyst (Wheeler et al., '40). The reactions were carried out at temperatures of 120–130°C. for triolein and 145–150°C. for tristearin, with a constant stream of dry nitrogen bubbling through the reaction mixtures. The triolein was purified by washing it in ether solution twice with 70% ethanol containing KOH and five times with 70% ethanol (Wheeler et al., '40). Tristearin was purified by crystallization from 95% ethanol.

Mono-olein and mono-stearin were prepared by the acetone-glycerol method (Malkin and Shurbagy, '36). To redistilled acetone-glycerol in solution in a dry quinoline and chloroform mixture were added slowly, with shaking and cooling, the respective acid chlorides. The reaction was permitted to proceed at room temperature for about 36 hours, and then the mixture was taken up in ether, washed, dried, and distilled to remove the solvent. The acetone complex was decomposed by cold HCl. The resultant mono-stearin was filtered and the precipitate was washed thoroughly with ice water. The mono-olein was taken up in ether for washing.

[2] Red oil, which is expressed from tallow fatty acids, contains 80% or more oleic acid.

[3] Eastman Kodak Co., C.P. Grade.

Mono-oleodistearin was obtained by the reaction of mono-olein with stearoyl chloride in solution in dry quinoline and chloroform. The reaction mixture was kept warm on a steam bath for 4 hours, then cooled and taken up in ether. The ether solution was washed with 0.5 N H_2SO_4 and with 5% K_2CO_3 solution, and then was dried and distilled to remove the larger portion of the solvent. Ethanol was added until there was a slight cloudiness and the glyceride was crystallized from solution at — 22°C., and dried in a vacuum desiccator. Mono-stearodiolein was prepared in a similar fashion from mono-stearin and oleoyl chloride.

Composition of diets

The basic diet used was as follows: crude casein, 18%; experimental fat, 15%; salt mixture, 7%; liver extract concentrate, 3%; brewers' type yeast, 1%; and dextrose, 56%. Four diets were prepared, differing only in the composition of the experimental fat: Diet A, 2 parts tristearin, 1 part triolein; diet B, 1 part tristearin, 2 parts triolein; diet C, distearo-mono-olein; and diet D, mono-stearodiolein. For the low fat diet, the above ratio of components was used, with no fat added.

Feeding methods and collection of feces

A group of five animals was started on each of the four diets. A 1-day orientation period was allowed on each diet, followed by an 8-day experimental period during which food consumption was measured and feces were collected. The feces were stored in methanol until analysed. At the end of the 8 days the animals were changed to a stock diet for 2 days, and then put back on the experimental diets again.

For the second experimental period the animals which had been on diet A were given diet C, and vice versa. The same was done with diets B and D. In this way it was possible to check each diet with two different groups of animals, and to compare the reaction of each group to two different types of glycerides (mixed glycerides vs. mixture of simple triglycerides) containing the same proportion of stearic and oleic acids. Unfortunately, we were able to continue the second period only so long as our diets lasted, which varied from 2 to 6 days. The data showing food consumption and fecal elimination are presented in table 1.

Extraction of fecal lipid

Total lipids were extracted and digestibility coefficients were calculated in approximately the same manner as was employed by Hoagland and Snider ('42). The feces were saponified in the methanol in which

they had been stored after mashing the fecal pellets. The saponification mixture was acidified with 35% H_2SO_4 and then extracted thoroughly with ether. The extracts were washed free of mineral acids with water, dried, and then the solvent was removed. The residues were evacuated at steam bath temperature until constant weight was attained.

From the weight of the acidic residue obtained in each case was subtracted the corresponding amount of lipid obtained on the low fat diet. The difference was multiplied by the factor 1.045 to convert to glyceride weight. The digestibility coefficients were determined from the calculated weight of excreted glyceride and the total amount of experimental fat ingested. These data are listed in table 1.

TABLE 1

Fat ingestion and excretion of rats on diets containing synthetic glycerides of stearic and oleic acids.

	EXPERIMENTAL DIETS								
	Low fat	*A*	*A*	*B*	*B*	*C*	*C*	*D*	*D*
Number of animal days	70	32	30	40	15	40	12	40	8
Food consumption (gm.)	..	340	528	583	239	628	163	596	97
Food per animal per day	..	10.6	17.6	14.6	15.9	15.7	13.6	14.9	12.1
Wt. fat in food (gm.)	..	51.0	79.2	87.5	35.9	94.2	24.5	89.4	14.6
Wt. feces (gm.)	124.1	91.4	124.1	113.5	40.4	119.8	25.6	114.7	15.4
Wt. acidic extract (gm.)	2.5	29.3	48.4	27.6	11.3	39.4	9.0	24.7	4.0
Corrected for low fat diet		28.2	47.3	26.2	10.8	38.0	8.6	23.3	3.7
Corrected for low fat diet × 1.045		29.5	49.4	27.4	11.3	39.7	9.0	24.3	3.9
Per cent of total fat undigested		57.8	62.4	31.3	31.5	42.1	36.7	27.2	26.7
Digestibility (%)		42.2	37.6	68.7	68.5	57.9	63.3	72.8	73.3
Digestibility based on combined periods		39.4		68.6		59.0		72.9	

TABLE 2

Analysis of lipid extracted from feces of rats on diet containing synthetic glycerides.

	EXPERIMENTAL DIET			
	A	*B*	*C*	*D*
Iodine value	12.3	23.8	18.0	28.5
Acid number	188.5	182.5	172.5	145.0
Wt. % palmitic	2.1	2.6	0.5	...
Wt. % stearic	81.8	67.8	78.2	65.8
Wt. % oleic	7.9	16.1	9.2	15.8
Wt. % non-saponifiable [1]	8.2	13.5	12.1	18.4

[1] Consists chiefly of distillation residue and material washed from column following distillation. Too dark for accurate saponification number, but probably contains up to 50% stearic acid.

Analysis of fecal lipid

The acidic fecal extracts from the two experimental periods were combined by groups and the iodine values and acid numbers were determined (table 2). Each combined extract was methylated and the mixed methyl esters were analyzed by the fractional distillation method for fat acid composition (table 2).

DISCUSSION

It is apparent from table 1 that there was very satisfactory agreement between the digestibility coefficients that were obtained during the first experimental period and the corresponding values for the second period. This agreement adds significance to the differences obtained between the digestibilities of the various experimental fats.

The results indicate rather clearly that stearic acid is very poorly digested, whether fed as a mixed glyceride or a simple triglyceride. Tristearin quite apparently is almost completely indigestible when mixed with triolein. When present in mixed glycerides, stearic acid becomes more indigestible as the amount of oleic acid is increased.

Inasmuch as it has been shown in previous work (Longenecker and Mattil, '42) that tristearin is partially utilized by the rat when it is the only fat in the diet, one is led to the thought that a degree of selective utilization may exist. That is, when tristearin is the sole fat in the diet, the rat is able to digest and metabolize stearic acid in order to fulfill partially, or perhaps completely, his energy requirements. When, however, a more desirable glyceride such as triolein is fed along with tristearin, the rat will attempt to meet his needs without stearic acid. Thus, it may be concluded that if the diet contained one of the customary edible fats, essentially all of the tristearin and most of the other stearic acid in the fat would not be metabolized, provided that the dietary requirements of the rat were met by the other more digestible fat acids.

The "solvent" action of oleic acid for stearic acid which tends to increase the digestibility of the latter is markedly greater with the mixed glycerides than with the mixtures of triglycerides. This may be due to the greater solubility of the mixed glycerides, as compared with tristearin, thus resulting in an increased rate of hydrolysis.

Consideration of these observations and of the ratios of stearic to oleic acids in the feces would seem to indicate that hydrolysis, or perhaps ester interchange, must precede absorption of glycerides as the general rule. If the fats were absorbed or rejected chiefly as the original triglycerides, one would expect to find approximately the same

stearic to oleic ratio in the feces as in the food in groups C and D. Such, however, is far from the case (table 2). The only alternative hypothesis which lends itself would be the complicated absorption → hydrolysis → resorption system, which seems the less probable of the two.

It is interesting to note that the amounts of oleic acid in the fecal lipids are in direct proportion to the amounts in the ingested fat (table 2). The explanation for this is not readily apparent, although it is more probably due to mechanical or physical factors rather than chemical or metabolic.

SUMMARY

Synthetic glycerides containing stearic and oleic acids have been prepared and incorporated into the diets of rats. The stearic acid in the glycerides has been shown to be very indigestible. It is better utilized when fed as mixed glycerides than when fed as tristearin mixed with triolein. The possibility of selective utilization of fat acids has been indicated. Support is given to the hypothesis that either hydrolysis of glycerides or ester interchange precedes absorption.

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STUDIES ON BONE FRACTURE HEALING

I. EFFECT OF VITAMINS A AND D [1]

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Interest in the relation between the healing of fractures and diet has been intensified by the war not only because of the increased incidence in the armed forces but also as a result of the dietary deficiencies of the civilian population. It is also a serious problem in those of advancing age for not only is delayed fracture healing more common in this group, but their diets are frequently lacking in essential food elements.

Excellent histological studies (Hertz, '36; Urist and McLean, '41) have demonstrated the effects of deficiencies of vitamins A, C, and D on the fundamental cellular processes involved in fracture healing. However, these studies are of necessity only qualitative.

The present investigation was undertaken to obtain quantitative data on the activity of calcification and the functional recovery in standard fractures of the fibulae of animals with experimentally produced dietary deficiencies. It was presumed that such quantitative methods might provide a means for detecting the effects of mild dietary deficiencies and so indicate the optimum level for fracture healing. They would also serve to test the effect of other measures designed to promote or delay fracture healing.

EXPERIMENTAL METHODS

Fractures of the middle of the right fibula (opposite the tibial spine) were produced in rats by the fracture forceps of Hertz ('36) or by operation. The fibula bears no weight, and is splinted naturally by the tibia so that no further care of the fracture is necessary. Animals were sacrificed at 4-day intervals over a period of 3 or 4 weeks. Twenty-four hours before each animal was sacrificed, it was given a dose of 0.2 mg. Sr^* [2] as the lactate by intraperitoneal injection. The Sr^* taken up by

[1] Aided by grants from the Nutrition Foundation, Inc., and from the Christine Breon Fund for Medical Research.

[2] The symbol Sr^* designates strontium "labeled" by the presence of its radioactive isotope.

the fractured fibula was measured directly with a Geiger-Müller counter, and compared with that taken up by the unfractured bone on the opposite side. The excess Sr^* in the fractured bone was taken as a measure of the calcification activity in the callus. This was expressed by the formula:

$$\text{Calcification Activity} = (\%\ \text{dose } Sr^*\ \text{in fractured bone} - \%\ \text{dose } Sr^*\ \text{in normal bone}) \times 10$$

Radio-autographs of fractured and control bones showed that the excess Sr^* in the fractured bone was localized in the callus. The radio-autographs were prepared by mounting the fractured and normal fibulae from typical animals on a rubber block in direct contact with No-Screen x-ray film. The radiations from the Sr^* produced a darkened image of the bone corresponding to the sites of active deposition.

The functional recovery was determined by measuring the breaking strength of both the fractured fibula, and of the unfractured bone on the opposite side. The apparatus used was a modification of that of Lindsay and Howes ('31) and was patterned after the standard engineering device for measuring the strength of beams. The bone is supported on the balance pan of a 1 kg. Toledo scale by two knife edges spaced 10 mm. apart. Pressure is exerted from above by two knife edges spaced equidistant from each other and from the supports. These are pivoted to adapt to any irregularity of the bone. In this way, the stress remains uniform between these points, over the central portion of the bone, including the site of the fracture. Pressure is increased at a uniform rate by rotation of a micrometer screw which presses down the upper knife edges. The actual load on the bone is read directly on the balance scale, and the load at which the bone breaks is taken as the breaking strength.

The functional recovery during healing is indicated by the increase in the breaking strength of the fractured bone, while the relative strength of the fracture as compared to the normal bone may be expressed by the ratio of the two breaking strengths; i.e.,

$$\text{Relative Fracture Strength} = \frac{\text{Breaking strength of fractured fibula}}{\text{Breaking strength of the unfractured fibula}}$$

RESULTS

Normal rats on stock diet

The rats used in this series were young adult animals 2 to 3 months old which had been reared and maintained on the standard stock colony diet. The results obtained from over ninety animals are summarized

in table 1. The figures given are the average values plus or minus the standard deviation.

In these normal animals, the calcification activity was very pronounced during the period from 8 to 16 days after the bone was fractured, corresponding to the primary calcification of the callus. The breaking strength of the fractured bone, insignificant at 4 days while the callus was soft, rose rapidly until by 12 to 16 days its value was comparable to that of the unbroken bone on the opposite side. Apparently in the rat, the fractured bone quickly attains and maintains a strength comparable to that of the normal bone.

TABLE 1

Fracture healing in normal rats on stock diet.[1]

DAYS AFTER FRACTURE	NO. OF RATS	WEIGHT	CALCIFICATION ACTIVITY IN % DOSE OF SR^* × 10			BREAKING STRENGTH		RELATIVE FRACTURE STRENGTH
			Fractured	Unfractured	"Callus"	Fractured	Unfractured	
		gm.				*kg.*	*kg.*	
4	7	175 ± 14	2.0 ± 0.5	1.5 ± 0.5	0.5 ± 0.3	0.00	0.53 ± 0.23	0.0
8	15	200 ± 26	3.7 ± 0.6	1.5 ± 0.5	2.2 ± 0.7	0.31 ± 0.13	0.55 ± 0.20	0.6
12	13	197 ± 25	5.4 ± 1.1	1.7 ± 0.4	3.7 ± 1.1	0.51 ± 0.12	0.63 ± 0.16	0.8
16	13	190 ± 27	4.1 ± 1.4	1.6 ± 0.4	2.5 ± 1.3	0.52 ± 0.14	0.58 ± 0.14	0.9
20	18	195 ± 24	3.0 ± 0.7	1.7 ± 0.5	1.3 ± 0.6	0.53 ± 0.18	0.67 ± 0.16	0.8
24	12	206 ± 21	2.9 ± 0.9	1.5 ± 0.7	1.4 ± 0.8	0.63 ± 0.18	0.75 ± 0.11	0.8
28	8	225 ± 40	2.5 ± 0.8	1.6 ± 0.7	0.9 ± 0.6	0.53 ± 0.21	0.66 ± 0.16	0.8
32	6	202 ± 32	2.5 ± 0.7	1.4 ± 0.2	1.1 ± 0.6	0.65 ± 0.20	0.68 ± 0.12	0.9

[1] Data given are mean values ± the standard deviation.

Effect of vitamin A deficiency

The observations of Mellanby ('41, '44), Wolbach and Bessey ('41), and Moore ('39) have established bone overgrowth as an important aspect of vitamin A deficiency. Mellanby believes that lack of vitamin A leads to increased activity of both the osteoblasts and osteoclasts of the bone with proliferation of cancellous bone at the expense of compact bone. Wolbach and Bessey, on the contrary, consider that vitamin A deficiency retards bone growth. Hertz ('36) observed that cartilage formation in the callus was inhibited in vitamin A deficient rats, although the fracture ossified and consolidated at the same time as in normal rats.

In view of these observations, and because of the frequent deficiency of vitamin A, particularly in war dietaries, fracture studies were carried out on vitamin A deficient rats both with and without vitamin A supplements. Rats were weaned at 3 weeks to the standard vitamin A

test diet (U. S. P. XI) and maintained on this diet for 5 weeks.[3] After the bones were fractured, the rats were divided into two groups, both of which were continued on the vitamin A deficient diet. The treated group received 10,000 units of vitamin A in shark liver oil at the time of fracture and at weekly intervals thereafter, while the untreated group received no supplement. The mortality among the untreated rats was high, and so the process of healing could not be followed beyond 16 days. The results obtained are summarized in table 2.

TABLE 2

Effect of vitamin A on fracture healing.[1]

DAYS AFTER FRACTURE	NO. OF RATS	WEIGHT	CALCIFICATION ACTIVITY IN % DOSE OF $Sr^{89} \times 10$			BREAKING STRENGTH		RELATIVE FRACTURE STRENGTH
			Fractured	Unfractured	"Callus"	Fractured	Unfractured	
		gm.				*kg.*	*kg.*	
			Vitamin A deficient diet					
8	14	146 ± 18	3.0 ± 0.7	1.7 ± 0.4	1.3 ± 0.5	0.24 ± 0.10	0.62 ± 0.09	0.4
12	11	140 ± 26	2.3 ± 0.7	1.4 ± 0.4	0.9 ± 0.5	0.30 ± 0.08	0.72 ± 0.11	0.4
16	12	129 ± 18	2.7 ± 0.6	1.5 ± 0.4	1.2 ± 0.4	0.44 ± 0.15	0.63 ± 0.11	0.7
			Vitamin A deficient diet plus 10,000 units of A per week					
8	11	168 ± 24	4.3 ± 0.6	2.7 ± 0.4	1.5 ± 0.8	0.41 ± 0.19	0.73 ± 0.12	0.6
12	10	167 ± 10	3.7 ± 0.7	2.1 ± 0.3	1.6 ± 0.6	0.54 ± 0.18	0.74 ± 0.10	0.7
16	10	161 ± 40	3.8 ± 0.6	2.4 ± 0.3	1.4 ± 0.6	0.54 ± 0.12	0.71 ± 0.19	0.8
20	6	174 ± 16	3.2 ± 0.6	1.8 ± 0.4	1.4 ± 0.3	0.59 ± 0.07	0.79 ± 0.11	0.8

[1] Data given are mean values ± the standard deviation.

The fracture callus of both treated and untreated groups was considerably smaller than that of the typical callus, and the "calcification activity" was less. However, the fracture strength of the treated rats, as indicated by the breaking strength, recovered just as quickly as it did in the control animals on stock diet. On the other hand, the recovery in breaking strength was significantly delayed in the untreated vitamin A deficient animals. It may be that this delay in fracture healing was occasioned by the general debility of these animals rather than any specific effect of the vitamin deficiency. It may be noted that no significant effect of vitamin A deficiency on the distribution and excretion of Sr^{89} was observed by us in tracer experiments.

Effect of vitamin D deficiency

The importance of vitamin D in the mineralization of bone has been established by numerous investigators. The healing process in the

[3] We are indebted to Mr. Mittler of the Laboratories of the California Packing Corporation for rearing the vitamin A deficient rats used in this investigation.

fracture callus of rachitic rats has been studied by a number of workers using histological methods (Hertz, '36; Compere, Hamilton and Dewar, '39; Urist and McLean, '41). These investigators found that rickets exerted a profound effect on the formation and mineralization of the fracture callus.

The effect of rickets was studied with our techniques in the following experiments. Rats were weaned at 24 days to the Rachitogenic Diet no. 2 (U.S.P. XII) and depleted for 25 days according to the usual technique.[4] It was necessary to produce the fractures of the fibula by operation because the bones were too soft for effective use of the fracture forceps. The animals were divided into two groups, both of which were maintained on the rachitogenic diet. One group received a supplement of 10 units of vitamin D (as irradiated ergosterol) per day, while the other group received no treatment. The results obtained are summarized in table 3.

The fibulae of all the rats were thin, poorly calcified, and pliable, so that it was difficult to obtain a clear-cut breaking strength measurement. The callus was large, soft, and cartilaginous. In the animals with severe rickets no appreciable deposition of Sr^{*} in the callus was evident either by direct measurement, or in radio-autographs. The breaking strength of the fractured bone was ill-defined due to the pliability of the bone, and while comparable to that of the unbroken bone on the opposite side, it was much less than that of the fibula of normal rats of the same age.

In those rachitic rats which received treatment some calcification did take place in the callus, but the calcification activity was much less than in normal rats, and the breaking strength resembled that of the untreated rachitic animals.

Effect of hypervitaminosis D

Because of the marked effect of toxic doses of vitamin D on calcium metabolism, and the frequent use of large doses of vitamin D in therapy, it was felt that a study of its effect on fracture healing might be of value. Grauer ('32) found that overdosage of vitamin D produces a stimulation of the fibrous layer of the periosteum through decalcification of the bone. Consequently there is a retardation of bone repair.

Young adult female rats 2 or 3 months old were used in the experiments. They were reared and maintained on the regular stock colony diet. After fracture had been produced in the usual way, they were given 40,000 units of vitamin D (as irradiated ergosterol) by mouth

[4] We are indebted to Mr. Theodore Sanford of the Booth Laboratories, Emeryville, for rearing the rachitic rats used in these investigations.

TABLE 3

Effect of vitamin D on fracture healing.[1]

GROUP	DAYS AFTER FRACTURE	NO. OF RATS	WEIGHT	CALCIFICATION ACTIVITY IN % DOSE OF $Sr^{85} \times 10$			BREAKING STRENGTH		RELATIVE FRACTURE STRENGTH
				Fractured	Unfractured	"Callus"	Fractured	Unfractured	
			gm.				*kg.*	*kg.*	
Severe rickets									
Ricketogenic	8	3	78 ± 6	0.7 ± 0.4	0.7 ± 0.5	0.0 ± 0.2	0.10 ± 0.02	0.17 ± 0.02	0.6
diet;	12	4	103 ± 2	0.7 ± 0.2	0.7 ± 0.3	0.0 ± 0.2	0.22 ± 0.08	0.23 ± 0.01	1.0
no supple-	16	2	87 ± 10	0.3 ± 0.1	0.5 ± 0.2	− 0.2 ± 0.1	0.19 ± 0.02	0.21 ± 0.05	0.9
ment	20	5	103 ± 18	0.4 ± 0.2	0.5 ± 0.2	− 0.1 ± 0.1	0.16 ± 0.07	0.22 ± 0.06	0.7
Treated rickets									
Ricketogenic	8	4	83 ± 21	1.6 ± 0.4	1.0 ± 0.3	0.6 ± 0.6	0.13 ± 0.01	0.16 ± 0.03	0.8
diet plus	12	3	72 ± 3	2.2 ± 0.5	1.4 ± 0.3	0.8 ± 0.5	0.19 ± 0.03	0.18 ± 0.03	1.1
10 units	16	4	98 ± 15	1.7 ± 0.5	0.9 ± 0.1	0.8 ± 0.5	0.20 ± 0.08	0.20 ± 0.09	1.0
vitamin D per day	20	4	85 ± 19	1.0 ± 0.1	0.7 ± 0.2	0.3 ± 0.2	0.15 ± 0.08	0.18 ± 0.05	0.8
Hypervitaminosis D									
Stock diet	8	5	168 ± 20	2.0 ± 0.4	0.8 ± 0.1	1.2 ± 0.4	0.12 ± 0.04	0.54 ± 0.05	0.2
plus 10,000	12	4	176 ± 23	1.4 ± 0.1	0.6 ± 0.2	0.8 ± 0.3	0.35 ± 0.16	0.68 ± 0.13	0.5
units vitamin D	16	3	143 ± 5	1.4 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.26 ± 0.04	0.50 ± 0.04	0.5
per day	20	3	173 ± 11	1.6 ± 0.3	0.8 ± 0.2	0.9 ± 0.3	0.29 ± 0.06	0.51 ± 0.06	0.6

[1] Data given are mean values ± the standard deviation.

every 4 days. The animals showed no toxic signs other than a loss of 5 to 10 gm. in weight per week. The results obtained are given in table 3.

In these rats, both the callus size and the calcification activity was much less than in the normal rats, and the recovery in breaking strength was greatly impaired.

SUMMARY

1. A method for studying healing in standard fractures of the rat fibula is described, in which calcification activity is determined by measuring the uptake of Sr^* by the callus, and functional recovery by the increase in breaking strength of the fractured bone.

2. In normal rats, the most active calcification in the callus occurs over the period from 8 to 16 days. The broken bone attains a strength comparable to that of the normal bone on the opposite side within 12 to 16 days.

3. In vitamin A deficient rats, the callus is smaller than in normal animals, and the calcification is less active. In those treated with large doses of the vitamin the increase in strength of the fractured bone was comparable to that in normal rats. On the other hand the untreated animals showed a significant delay in fracture healing. This may have been due to the debilitated condition of the latter.

4. In rachitic animals, there is no significant calcification of the callus, unless vitamin D is added to the diet.

5. In animals receiving toxic doses of vitamin D, the callus is small, calcification activity is reduced, and recovery in strength is delayed.

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THE EFFECT OF SOY FLOUR ON THE NUTRITIVE VALUE OF THE PROTEIN OF WHITE BREAD[1]

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ONE FIGURE

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Previous studies have shown that the addition of 15 or 20% of soy flour significantly improves the protein quality of bread, but the product is so different from white bread that it is not accepted by the average consumer. Since 5% of soy flour can be used without appreciably altering the appearance or flavor of white bread it seemed of interest to determine whether such an addition would improve the protein quality.

This paper reports such a study with rats, using bread made from enriched patent flour and containing 3% of whole milk solids. Data are also presented on the milk solid content of a variety of types of national brands of breads purchased on the open market.

Jones and Divine ('44) reported the results of supplementing patent flour with different amounts of soy flour. The gain per gram of protein consumed was 0.75 gm. for the patent flour; 1.38 gm., 2.16 gm., and 2.27 gm. when 5%, 10%, and 15%, respectively, of the soy flour was added. Johns and Finks ('21) found that rats fed wheat bread gained only 1.0 gm., whereas on wheat-soy bread they gained 1.5 gm. for each gram of protein consumed. Kon and Markuze ('31) showed a similar improvement in the protein quality of bread by the addition of soy flour. In rat growth studies, they found that the average gain per gram of protein consumed was 1.09 gm. for wheat bread and 1.62 gm. when soy flour was added.

Mitchell and Carman ('26) reported a biological value of 50 for patent flour. Klein et al. ('26) reported an average biological value of 42 for patent flour. Fairbanks ('38, '39) in comparing breads containing 0, 6 and 12% non-fat milk solids found that increasing the milk solids improved the palatability and the growth-promoting value of the bread when fed to rats. Henry et al. ('41) reported biological values of 44.7 for water bread, 47.6 for 2% milk bread, and 49.7 for 6% milk bread.

[1] This study was supported in part by funds from a grant of the Edward A. Filene Good Will Fund, Inc.

Mitchell et al. ('43) and Light and Frey ('43) have shown that 6% dried skimmilk solids in bread results in growth equal to that produced by whole wheat bread. Murlin et al. ('41) observed that although white bread has a higher true digestibility by human subjects, its relative value may be lower than a peeled wheat or whole wheat bread. They reported the following digestibility and relative values: lean white bread, 99.4 and 75.3; peeled wheat bread, 94.9 and 77.9; whole wheat bread (with 5% non-fat milk solids) 92.8 and 77.8 respectively.

STUDIES WITH RATS

Growth studies have been carried out with rats fed diets containing white bread, white bread with 5% soy flour (flour made from the soybean, Glycine hispida), patent flour, and patent flour with 5% soy flour. The biological value of the protein of white bread and bread with 5% soy flour was also measured.

Weanling albino rats fed the diets shown in table 1 were paired according to sex, weight, and litter. Each group used in the growth studies

TABLE 1

Composition and protein contents of the diets.

INGREDIENT	DIET NUMBER			
	1	2	3	4
	%	%	%	%
Patent flour	....	....	77.52	73.64
Soy flour (full-fat)	....	....	....	3.88
White bread	74.40	....	....	....
Wheat-soy bread	....	66.66	....	....
Sucrose	7.60	15.34	4.48	5.48
Butterfat	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	4.00
Salt mixture[1]	4.00	4.00	4.00	4.00
Cellophane	4.00	4.00	4.00	4.00
Protein content ($N \times 5.7$)	9.2	9.2	9.1	10.3

[1] Osborne and Mendel.

consisted of five males and five females. The animals were weighed twice each week during the 6-week period. The food was weighed daily and the food intakes were equalized. Scattering of the diets was slight but was taken into consideration when it occurred. A rice polish concentrate and crystalline riboflavin supplied daily approximately 30 μg. thiamine, 400 μg. nicotinic acid, 30 μg. pyridoxine, 55 μg. pantothenic acid, 5.4 μg. filtrate factor, 40 μg. riboflavin. Two drops of cod liver oil

were fed three times each week. Distilled water was kept before the animals at all times.

The dough for the two breads [2] included a basal mixture containing whole milk solids 19.8 gm., yeast 19.8 gm., sucrose 26.4 gm., salt 13.2 gm., shortening 13.2 gm., and water 396 ml. To these ingredients were added 660 gm. of wheat flour for the white bread, and 627 gm. wheat flour and 33 gm. full fat soy flour [3] for the wheat-soy bread mix. The white bread contained 13.5% protein and the wheat-soy bread 14.9% protein on a dry matter basis.

RESULTS

The starting weight, gains, food consumption, protein intakes and the protein efficiencies for the rats fed the bread diets are shown in table 2. The rats receiving the wheat-soy bread diet in nine cases out of ten gained more weight than those fed the white bread diet. The difference in weight gains of the rats fed these bread diets is highly significant, odds 999:1. The grams of gain per gram of protein consumed were 20% higher for the wheat-soy bread than for the white bread diet.

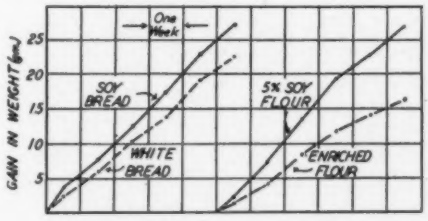

Fig. 1 Cumulative gain of rats fed white bread and white bread with 5% soy flour, and of those fed enriched patent flour with and without 5% soy flour.

Similar studies comparing the flours only (diets 3 and 4, table 2) gave average protein efficiencies of 0.80 gm. for white flour and 1.13 gm. for white flour plus 5% soy flour. Odds favoring significance of the difference between these average values were 4999:1.

A comparison of the progressive gains of the rats presented in figure 1, emphasizes the fact that the soy flour improved the growth promoting value of the diets and that the presence of 3% of whole milk solids

[2] We acknowledge the assistance of Miss Catherine Personius, Professor of Home Economics, in making the breads.

[3] Sold by Soya Corporation of America, New York, N. Y.

did not mask that improvement. The difference between the diets became increasingly greater as the experiment progressed.

Biological values. The biological values of the two breads were determined by the method of Mitchell ('43), using diets 1 and 2 (table 2) fed in the growth studies. During the basal period a low protein egg diet was fed. This diet contained 3.95% protein and was composed of the following ingredients: egg solids 7.8, cooked starch 69.2, sucrose 5.0, butterfat 5.0, soybean oil 5.0, O & M salt mix 4.0, and cellophane 4.0%.

Twelve male albino rats weighing 43 to 53 gm. were paired according to litter weight. The food intake of the pair-mates were equalized.

TABLE 2

Summary of the results of the growth study in which bread diets were fed.

RAT NO.	SEX	DIET [1]	STARTING WEIGHT	WEIGHT GAINS	FOOD INTAKE	PROTEIN INTAKE	GRAMS GAIN PER GRAM PROTEIN
			gm.	*gm.*	*gm.*	*gm.*	
21	Male	White bread	40	17	201	18.49	.92
31	Male	Wheat-soy bread	39	19	202	18.58	1.02
22	Male	White bread	44	26	265	24.38	1.07
32	Male	Wheat-soy bread	44	35	265	24.38	1.44
23	Male	White bread	47	24	255	23.46	1.02
33	Male	Wheat-soy bread	46	28	255	23.46	1.19
24	Male	White bread	45	16	207	19.04	.84
34	Male	Wheat-soy bread	47	16	207	19.04	.84
25	Male	White bread	54	22	282	25.94	.85
35	Male	Wheat-soy bread	56	26	283	26.04	1.00
26	Female	White bread	40	24	223	20.52	1.17
36	Female	Wheat-soy bread	39	29	223	20.52	1.41
27	Female	White bread	44	26	274	25.21	1.03
37	Female	Wheat-soy bread	42	28	274	25.21	1.11
28	Female	White bread	43	27	265	24.38	1.11
38	Female	Wheat-soy bread	43	31	265	24.38	1.27
29	Female	White bread	48	20	292	26.86	.74
39	Female	Wheat-soy bread	46	32	292	26.86	1.19
30	Female	White bread	50	27	289	26.59	1.02
40	Female	Wheat-soy bread	50	32	289	26.59	1.20
Average		White bread	45.5	22.9	255.3	23.49	.97
Average		Wheat-soy bread	45.2	27.6	255.5	23.51	1.17

[1] Both diets contained 9.2% protein.

The same vitamin supplements were given throughout this study as during the growth studies. A 6-day preliminary period of constant food intakes preceded each collection period. Collections of urine and feces were made for 7 days on each diet. The test diets were fed during the first period. The basal diet was fed during the second period. During the third period the test diets were reversed and again fed. The biological values obtained by this study are shown in table 3. The average value for wheat-soy bread is approximately 10% higher than the value for white bread. The mean difference between the biological values of these bread proteins is highly significant, odds 249 : 1.

TABLE 3

Biological value of the protein of white bread and soy bread.

BREAD	PAIR NUMBER						AVERAGE
	1	2	3	4	5	6	
White bread	41.9	38.4	49.2	41.8	41.9	49.8[1]	43.3
Wheat-soy bread	45.4	49.4	48.3	48.2	46.0	56.4[1]	47.7

[1] One urine sample was lost. Therefore, these represent a single value instead of an average of two values.

TABLE 4

Non-fat milk solids in bread purchased on the open market (dry basis).

TYPE OF BREAD	NUMBER OF SAMPLES	NON-FAT MILK SOLIDS	
		Range	Average
		%	%
White	11	1.83–4.29	3.07
White (milk solids)	8	0.35–6.20	2.78
Wheat breads[1]	8	0.34–2.49	1.30
Wheat breads (milk solids)[1]	6	0.56–3.07	1.62

[1] Includes breads labeled as wheat, cracked wheat, 75% whole wheat and 100% whole wheat.

Chemical analyses of breads for milk solids. Thirty-three samples of breads, mostly national brands were collected on the open market in July and August of 1942 and analyzed for milk solids. This was previous to the publication of Food Distribution Order no. 1 (Federal Register '42), requiring that white bread contain not less than 3 nor more than 4% of milk solids, which became effective January 18, 1943. The breads were obtained from the Ithaca, New York, market with the exception of four samples, two of which came from Kansas and two from Missouri. Milk solids were determined by the method of Magraw and Copeland ('36). The results are shown in table 4.

Only three of thirty-three breads examined contained as much as 6% of non-fat milk solids, although that level has frequently been quoted by bakers as being a desirable amount to include in white bread. The average non-fat milk solids for breads claiming milk as a constituent amounted to 2.28% of the dry bread, while the brands not claiming milk contained an average of 2.32%. The average for all brands was 2.30%, and approximately one-fifth of them contained less than 1%.

SUMMARY

Growth studies and biological values are reported which show that the addition of 5% soy flour significantly improves the growth promoting value of white bread which contains 3% of whole milk solids.

White bread containing 3% of milk solids gave 0.97 gm. gain per gram of protein consumed and bread with 5% soy flour, containing an equal amount of milk, 1.17 gm.; the difference is significant. The biological value of the protein of the white bread as determined by the Mitchell method was 43.3 as compared with 47.7 for the bread with 5% soy flour.

The analysis of thirty-three samples of breads purchased in 1942 showed a range from 0.34 to 6.20% in the content of milk solids (dry basis) and an average value of 2.30%. Breads labelled as containing milk varied as widely and contained no more milk solids than breads not so labelled.

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THE SULFUR BALANCE OF THE NON-LAYING, MOLTING AND LAYING HEN [1]

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ONE FIGURE

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Although sulfur in the reduced form is, like phosphorus and nitrogen, a normal constituent of the hen's egg, little or no attention has been given to the metabolism of this element in the hen or to requirements during laying and non-laying states. Supplements of cystine on a nitrogen-free diet were shown by Ackerson and Blish ('26) to have a sparing effect upon the endogenous nitrogen metabolism of the molting hen. A study of the loss or retention of sulfur on a laying ration, made up of commonly used ingredients, during periods of molting and egg production should give clues concerning the adequacy of the diet during each of these phases and possibly concerning any special needs for sulfur. The present investigation was planned to determine the sulfur balance of the hen during molting and during periods of low and high egg production.

EXPERIMENTAL

Four hens were used for the study. Each bird was housed in an individual cage with a mesh floor and fitted with a feeding cup designed for the measurement of feed consumption. The ration used consisted of 1 part ground wheat, 1 part ground corn, and 2 parts of a practical laying mash which had the following percentage composition: yellow corn 21, wheat middlings 20, wheat bran 20, meat scrap (55% protein) 15, pulverized oats 10, alfalfa meal 5, dried skimmed milk 5, calcite flour 2, salt 1 and cod liver oil 1.

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The excreta were collected on a glass plate, dried at room temperature with the aid of an electric fan, and ground in a Wiley mill. Charcoal or carmine was used as a marker in making the collections of excreta. Feathers were collected and clipped into fine pieces before analysis. A few feathers were shed during many of the collection periods, but only when the loss exceeded 0.5 gm. per day were the birds considered to be in a molt. Eggs were dried rapidly with a fan and ground in a mortar.

The oxygen bomb method of combustion used by Marston ('38) was chosen to bring about the destruction of organic matter and to convert reduced sulfur to the sulfate form. In early trials it was noted that a small quantity of incompletely burned material adhered to the nickel pan. This was prevented by lining the pan with ashless filter paper which served as an insulating material. The light bulky nature of feathers necessitated special precautions. After being clipped into small pieces, the weighed sample was wrapped in ashless filter paper, soaked in a solution of cellulose acetate in acetone and ethyl acetate and dried in an oven. This treatment bound the feathers in a compact mass and prevented scattering of the partly burned pieces during combustion.

An attempt was made to use the tetrahydroxyquinone titration method described by Brunjes and Manning ('40), for the determination of sulfate after the combustion of feed, excreta, or eggs. The high phosphate content of these materials, however, interfered with the determination of sulfate. Consequently, the A.O.A.C. gravimetric procedure was followed ('40). The tetrahydroxyquinone titration method was used for feathers because they did not contain enough phosphate to interfere.

When the hens had become accustomed to the confinement and the ration, a test period of approximately 3 months was begun. During this time the hens ceased laying, passed through a period of molting and then resumed egg production. Nine collections of 4 or 12 days' duration were made for each hen. These included periods of low egg production, molting and high egg production.

RESULTS AND DISCUSSION

As an example of the type of data obtained, the results of four balance periods with hen 1 are presented in detail in table 1. The four periods are representative of the effect on the sulfur balance of feather loss during molting, of no egg production, and of low and high egg production. The data of the thirty-five balance periods are summarized in figure 1.

TABLE 1

Average daily sulfur balances of representative periods.

	HEN 1			
	Period 3 (4 days)	Period 5 (4 days)	Period 11 (12 days)	Period 13 (12 days)
Body weight, gm.	1500	1500	1780	1870
Feed consumed, gm.	47	86	93	72
Sulfur in feed, %	0.167	0.169	0.170	0.169
Sulfur intake, mg.	78	145	158	122
Weight of air-dried droppings, gm.	18.8	25.5	27.0	22.0
Sulfur in droppings, %	0.343	0.324	0.357	0.416
Sulfur in droppings, mg.	64.0	82.0	96.5	92.0
Egg production rate, %	0	0	16	58
Dry weight of eggs, gm.	..	..	2.6	9.9
Sulfur in eggs, %	..	..	0.444	0.481
Sulfur in eggs, mg.	..	..	11.5	48.0
Weight of feathers, gm.	1.2 (molting)	0	0	0
Sulfur in feathers, %	2.09	..	..	..
Sulfur in feathers, mg.	25	..	..	..
Sulfur output, mg.	89	82	108	140
Daily sulfur balance, mg.	− 11	+ 63	+ 50	− 18

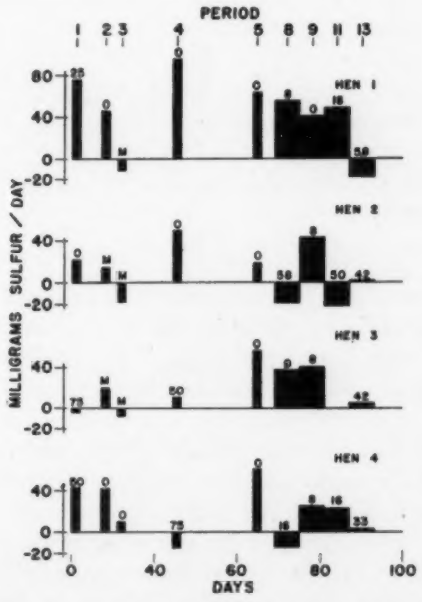

Fig. 1 Sulfur balances of hens. Numbers above the bars indicate the percentage of egg production. M indicates periods of molting in which the feather loss exceeded 0.5 mg. per day. Periods 1–5 were of 4 days' duration, periods 8–13 of 12 days' duration.

Period 1 (fig. 1), October 14th–18th, represents the close of egg production, periods 2 and 3 occurred during molting, and periods 4, 5, 8, 9, 11 and 13 represent a resumption and gradual increase of egg production. The study was terminated on March 8th. During molting the degree of sulfur retention was determined by the extent of feather loss. In some cases the sulfur balance was markedly negative and of the same order of magnitude as that which occurred during periods of high egg production. Thus for hen 2, period 3, the average daily balance was — 18 mg., which is of the same order as that observed later for the same hen when egg production was 58% and 50%. (Maximum

TABLE 2

Average daily sulfur balance and average daily sulfur excretion at various levels of egg production.

EGG PRODUCTION [1]	AVERAGE DAILY S BALANCE	AVERAGE DAILY S EXCRETION	NUMBER OF OBSERVATIONS
%	mg.	mg.	
0	+ 44.0	97	12
0 (molt)	— 0.6	81	5
8	+ 40.8	98	4
16	+ 19.7	121	3
25	+ 76.0	83	1
33	+ 0.2	100	1
42	+ 3.5	73	2
50	+ 10.6	93	3
58	— 18.0	93	2
75	— 11.0	98	2

[1] Maximum egg production potentiality is considered to be one egg per day. The number of eggs produced divided by the number of days in the collection period times 100 is the percentage egg production.

egg production potentiality is considered to be one egg per day. The number of eggs produced divided by the number of days in the collection period times 100 is the percentage egg production.) The sulfur balances for these production levels were — 22 mg. and — 18 mg., respectively.

Although there was no close direct relationship between egg production and the daily sulfur balance (table 2), the average daily sulfur balance was found to be + 20 mg. or higher for levels of egg production of 25% and less. When production was greater than 25%, the balance was of the order of + 10 mg. or less, becoming negative for production rates higher than 50%. To maintain a hen in positive sulfur balance at high levels of egg production, the ration would have to contain a

higher percentage of sulfur-containing substances or the feed consumption would have to be increased.

Even when sulfur demands are large, as in high egg production, the sulfur in the droppings, expressed as a percentage of that consumed, remains high. Thus in seven periods in which egg production was 50% or higher, the average value was 64%, in 13 periods of nonproduction 71%, and in 5 periods of molting 80%. That sulfur compounds are not retained to maintain sulfur equilibrium may be due to metabolic processes, such as methylation by methionine, in which the excretion of the demethylated portion of the methionine molecule, or degradation products from this portion, occurs. Another possibility is that the body has absorbed from the feed sulfur-containing compounds which are of no value in metabolic processes, egg production, or feather formation and are therefore excreted. A third possibility is that certain sulfur compounds are not absorbed and become a part of the fecal portion of the droppings.

SUMMARY

1. The sulfur balance of the hen has been determined during low and high egg production and during molting.

2. With an increase in egg production the sulfur balance decreased, becoming negative in most cases when the production rate went above 50%.

3. During the feather loss of the molting period, the sulfur balance decreased and became negative when feather loss was heavy.

4. Sulfur in the droppings, calculated as a percentage of that in the feed, remained high even during severe feather loss or high egg production.

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AVAILABILITY OF VITAMINS IN FOODS AND FOOD PRODUCTS

II. RIBOFLAVIN BALANCES IN DRIED LIVER, IN A LIVER VITAMIN CONCENTRATE, AND IN BREWERS' AND BAKERS' YEAST [1]

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In the first paper of this series (Sure, '44a) it was shown that thiamine in three brands of brewers' yeasts of various potencies is well utilized but there was 6 to 11% poorer absorption of thiamine in one type of yeast compared with an equivalent intake of pure vitamin B_1.

In this communication results of riboflavin balance studies are reported on dehydrated liver, on dehydrated vitamin concentrate prepared from liver,[2] on three brands of dried brewers' yeasts, and on one brand of dried bakers' yeast.

EXPERIMENTAL

In order to further reduce the possibilities of bacterial synthesis in the fecal excretions (Sure and Ford, '42), the feces were collected twice daily, at 8 A. M. and at 4:30 P. M., then covered with petroleum ether in amber colored bottles and were kept in an electric refrigerator at about 30°F. for a week until they were ready for analysis. For the riboflavin content of feces we used the procedures of Conner and Straub ('41) and for the riboflavin content of urine we employed our recent modifications (Sure, '44b) of the method of Hodson and Norris ('39) for determining the riboflavin content of foodstuffs.

The riboflavin content of the dried liver was 60 μg./gm. and of the liver vitamin concentrate, 225 μg./gm. Of the dried liver, 61% of the riboflavin was found to be in the free form. Of the liver vitamin concentrate, 72% was found to be in the free form. The riboflavin content of brewers' yeasts A, B, C and the bakers' yeast were 50, 70, 40, and

[1] Research paper no. 798 Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

[2] Kindly furnished by Dr. David Klein of the Wilson Laboratories, Chicago, Ill.

46 μg./gm., respectively. Approximately 50% of the riboflavin in the yeasts was in the free form.

The animals were fed a purified synthetic diet of the following percentage composition: vitamin-free casein, 18; cellu-flour, 2; salts no. 1 (Sure, '41), 4; butter fat, 5; and cerelose, 71. The vitamin supplements were given daily separately from the ration, as follows: 20 μg. thiamine, 20 μg. pyridoxine, 6 mg. choline chloride, and 200 μg. calcium pantothenate. As a source of vitamins A and D, three drops of halibut liver oil were given once weekly to each animal. The daily doses of dried liver products and dried yeasts were administered in petri dishes.

For the riboflavin metabolism studies two groups of male rats, twenty-four in each set, were used. The animals of the first group were started on experiments when 54 days of age, weighing 85 to 124 gm. each. The animals of the second set were placed on experiments when 31 days old, weighing 72 to 88 gm. each. The first set was depleted of riboflavin for 64 days and the second set for 34 days. The period of depletion was determined by the characteristic signs of riboflavin deficiency, such as keratitis, cataracts, alopecia, and muscular incoordination. During the depletion periods two animals of the first set and one of the second set died. It was found in this investigation that the same animals could be used for balance studies of several foods. After the animals were well depleted of riboflavin, data on urinary and fecal excretions of this vitamin were secured, which were subtracted from results obtained following the administration of dehydrated food-products. The animals were then ready for riboflavin balance studies.

The results of this investigation are submitted in summarized form in table 1.

Liver

Riboflavin balance studies were carried out on twenty-two animals for 21 days, allowing 20 μg. of this vitamin daily. A daily dose of 333 mg. of the dried liver, which furnished 20 μg. riboflavin, was administered to ten animals. At the same time an equivalent amount of pure crystalline riboflavin was given daily to twelve other animals. The next set of rats received 40 μg. riboflavin daily, which brought the dose of dried liver to 666 mg. Twelve rats received 40 μg. of pure riboflavin daily and 11 animals were given 666 mg. dried liver daily which furnished the same amount of this vitamin. The latter metabolism experiments were continued for 14 days. It will be noted that the fecal riboflavin excretions of the animals on the dried liver were two and one-half to three times as great and the urinary riboflavin excretions

TABLE 1

Riboflavin balances in dried liver, in a liver vitamin concentrate, and in brewers' and bakers' yeasts.[1]

FOOD PRODUCT OR PURE RIBOFLAVIN	NO. OF ANIMALS	METABOLISM PERIOD	DAILY RIBOFLAVIN INTAKE	TOTAL RIBOFLAVIN INTAKE	CHANGE IN BODY WEIGHT	RIBOFLAVIN EXCRETED IN URINE[2]		RIBOFLAVIN EXCRETED IN FECES[2]	
		days	*μg.*	*μg.*	*gm.*	*μg.*	*% of T. I.*[3]	*μg.*	*% of T. I.*[3]
Dried liver	10	21	20	420	+ 76.6	44.9	10.7	177.4	42.2
Pure riboflavin	12	21	20	420	+ 57.2	24.8	5.9	70.4	16.8
Dried liver	11	14	40	560	+ 65.2	72.2	12.8	221.1	39.4
Pure riboflavin	12	14	40	560	+ 50.5	28.1	5.2	67.7	12.1
Dried liver vitamin concentrate	12	14	20	280	+ 11.9	44.4	15.9	173.6	62.0
Pure riboflavin	10	14	20	280	+ 15.7	33.7	12.3	64.8	23.1
Dried liver vitamin concentrate	12	14	40	560	+ 13.4	43.1	7.7	81.6	14.4
Pure riboflavin	11	14	40	560	+ 15.9	78.7	14.1	269.0	48.1
Brewers' yeast A	6	21	20	420	+ 56.0	39.0	9.0	127.0	30.2
Brewers' yeast B	6	21	20	420	+ 57.0	38.5	9.0	91.4	21.8
Brewers' yeast C	6	21	20	420	+ 48.5	54.8	13.0	178.4	42.5
Bakers' yeast	6	21	20	420	+ 53.0	57.8	13.8	170.9	40.7
Pure riboflavin	10	21	20	420	+ 40.0	47.1	11.2	107.1	25.5

[1] Figures in this table represent averages per animal.
[2] Corrected for the amount excreted on a riboflavin deficient ration.
[3] T. I. = Total intake.

two to two and one-half as great as the excretions of the animals which received the same daily dose of pure vitamin.

Yeasts

All the yeasts were fed daily in amounts which provided 20 μg. riboflavin, as follows: brewers' yeasts: A, 400 mg.; B, 286 mg.; C, 500 mg.; and bakers' yeast, 435 mg. The metabolism period was 21 days. From table 1 it is apparent that the animals on the various types of yeasts varied considerably in the fecal excretions of riboflavin, the significance of which will be discussed later in the paper. There was little change, however, in the urinary excretions of riboflavin during the corresponding periods on the various yeasts.

Liver vitamin concentrate

The dried vitamin concentrate prepared from liver was fed in daily amounts of 89 and 178 mg., which provided 20 and 40 μg. riboflavin,

respectively. Equivalent amounts of pure riboflavin were fed to about the same number of animals. It is evident from table 1 that the animals which received the riboflavin from the liver vitamin concentrate excreted about three times as much of this vitamin in the feces as the rats which received the same amounts of the pure vitamin. There were also greater excretions of riboflavin in the urine in the animals which ingested the liver vitamin concentrate than in the rats which received the equivalent amounts of pure vitamin but the differences were not as marked as in the fecal excretions.

DISCUSSION

Since this study was completed the results of the work of Mannering, Orsini and Elvehjem ('44) appeared which demonstrated that the nature of the carbohydrate markedly influences the fecal riboflavin excretions. The substitution of 40% sucrose by an equivalent amount of lactose in the diet almost quadrupled the weekly riboflavin output. The authors attribute the increased fecal riboflavin excretions to bacterial synthesis in the intestinal tract.

The question arises whether the large increased excretions of fecal riboflavin following the feeding of the dried liver and the dried liver vitamin concentrate are of dietary or of bacterial origin. If the large excretions of fecal riboflavin on the liver products are due to poor absorption, then it would follow that the riboflavin in these food products is present in a combined form which is largely unavailable to the animal organism. However, as stated earlier in the paper, it was found by analysis that the riboflavin in these liver products is present mainly in the free form; hence, poor absorption is not the main factor contributing to the large fecal riboflavin excretions; these high excretions are probably due to a large extent to bacterial synthesis.

With regard to the large fecal excretions following the administration of various yeasts, two explanations are possible, particularly, since yeasts vary greatly in composition, depending on strain and the culture medium on which they are grown. Either yeasts contain variable constituents which may influence bacterial synthesis in the gut, thus resulting in greater fecal riboflavin output, or the latter may be due to poor absorption (50% of the riboflavin in yeasts being in combined form) because of the changes being produced in the yeasts by the method of dehydration.

No noteworthy differences were found in urinary excretions of riboflavin in the animals which secured this vitamin from the yeasts as

compared with those which received the equivalent amounts of the pure vitamin. However, the rats on the dried liver and on the higher intake of liver vitamin concentrate excreted larger amounts of riboflavin in the urine than did the animals given the same amounts of the pure vitamin. The reason for this is not apparent, since definite information on absorption is lacking because of the complication of bacterial synthesis.

The greater gains in body weight of the animals which received the liver products and yeasts compared with the rats which received the synthetic vitamin B complex mixture may be due to the presence in the natural food-products of unidentified growth-promoting essentials. p-Aminobenzoic acid and inositol were not included in the mixture of the B vitamins, because they were found non-essential for growth of the rat in our laboratory (Sure, '43). Biotin and folic acid were not available.

SUMMARY

Albino rats fed dried liver and a dried vitamin concentrate prepared from liver as sources of riboflavin excreted much greater proportions of the total intake of this vitamin in the feces and urine than animals which were given equivalent amounts of this vitamin. Since the greater part of the riboflavin in these liver products exists in the free state, the large excretions of this vitamin in the feces could not be due to poor absorption; in all probability they were due to bacterial synthesis. In the yeasts about 50% of the riboflavin was present in the free form; therefore, the large fecal excretions of riboflavin of the animals which received the yeasts may have been of dietary or of bacterial origin.

The animals on the dried liver and on the higher intake of liver vitamin concentrate excreted larger amounts of riboflavin in the urine than the animals given the same amounts of the pure vitamin. The reason for this is not at all clear, since we have no definite information on absorption because of the complication of bacterial synthesis.

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